AUTONOMIC INFLUENCES ON THE PRODUCTION OF CEREBROSPINAL FLUID

By

JOSEPH ROSCOE HAYWOOD

A DISSERTATION PRESENTED TO THE
GRADUATE COUNCIL OF THE UNIVERSITY OF FLORIDA
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE
DEGREE OF DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA
1976

To Carol

for her understanding, patience, and love

To Becca and Alyson

for giving me so much happiness

ACKNOWLEDGMENTS

I wish to express my sincere gratitude to Dr. Betty P. Vogh for her guidance throughout this course of study. She has been most helpful as a teacher, as a researcher, and as an advisor, but especially as a friend whose interest in her students extends beyond science.

I am also grateful to the other members of my supervisory committee, Dr. C.Y. Chiou, Dr. Carl Feldherr, Dr. David Silverman, and Dr. David Travis for their contributions. The advice of Dr. Thomas Maren, Dr. Peter Lalley, Dr. Sidney Cassin, and the faculty of the Department of Pharmacology and Therapeutics is also greatly appreciated.

I also thank Mr. Mark Loveland for his friendship, sense of humor, and technical assistance and Ms. Joyce Simon for her support and contribution in the preparation of this manuscript.

My sincere thanks are also extended to my fellow graduate students for their encouragement and friendship during these years.

This research was supported by a traineeship from the National Institutes of Health (GM-00760) and a research grant from the National Institute of General Medical Sciences (GM-16934).

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

KEY TO ABBREVIATIONS

CBF	Cerebral Blood Flow
CSF	Cerebrospinal Fluid
cpm	Counts per minute
cAMP	Adenosine 3',5' cyclic monophosphate
cGMP	Guanosine 3',5' cyclic monophosphate
ED_{50}	Median effective dose (dose to produce 50% effect)
gm	Gram
HC-3	Hemicholinium-3
ITP	1-isopropylamino-3(2 thiazoloxy)-2 propanol HCl
kg	Kilogram
µg	Microgram
µl	Microliter
mg	Milligram
ml	Milliliter
mm	Millimeter
mM	Millimolar
msec	Millisecond
min	Minute
M	Molar
M.W.	Molecular weight
sec	Second
Phe	Phenylephrine

Abstract of Dissertation Presented to the Graduate Council of the University of Florida in Partial Fullment of the Requirements for the Degree of Doctor of Philosophy

AUTONOMIC INFLUENCES ON THE PRODUCTION OF CEREBROSPINAL FLUID

By

Joseph Roscoe Haywood

December, 1976

Chairperson: Betty P. Vogh
Major Department: Pharmacology and Therapeutics

For the first time, treatments which stimulate or inhibit autonomic nervous system receptors were tested for their effects on cerebrospinal fluid (CSF) production using the dilution of blue dextran during ventriculo-cisternal perfusion in cats. Initial control CSF formation ranged from 15 to 25 µl/min and gradually decreased at the rate of 0.015 µl/min each minute between 60 and 300 minutes.

The cholinergic agonist, carbachol, in single intravenous injections caused a dose-dependent increase in the production of new fluid. The maximum increase was 10.6 ± 2.1 µl/min which was blocked by atropine.

Single intravenous injections of the alpha adrenergic agonist, phenylephrine, also increased CSF production to a maximum of 10.6 ± 1.5 µl/min over control. This dose-dependent effect was prevented by phentolamine and by atropine. An increase in CSF formation during infusion of phenylephrine was blocked by administration of hemi-cholinium-3 through a reduction of endogenous

acetylcholine. The ability of atropine and hemicholinium to inhibit the increase in CSF production by phenylephrine demonstrates that the alpha adrenergic agonist probably activates a cholinergic pathway. Atropine or phentolamine alone reduced CSF formation 2.6 ± 0.5 and 3.7 ± 0.8 µl/min, indicating that the cholinergic pathway exerts some control over normal fluid production. Neither atropine nor phentolamine was able to add to the maximal reduction in CSF formation caused by the carbonic anhydrase inhibitor methazolamide.

The $beta_2$ adrenergic agonist, salbutamol (albuterol), also increased the rate of CSF formation, but it was only half as effective as carbachol and phenylephrine. The $beta_1$ adrenergic agonist, 1-isopropylamino-3(2 thiazoloxy)-2 propanol HCl (ITP), had no effect on CSF production. Propranolol did not affect fluid formation but did reduce the effect of salbutamol.

Bilateral stimulation of the cervical sympathetic trunk reduced CSF production 3.0 ± 0.6 µl/min. This observation indicated that alpha adrenergic agonists probably act at the choroid plexus to decrease CSF formation; however, any direct action of circulating alpha agonists is masked by activating the cholinergic pathway.

Experiments were also performed to determine whether autonomic agents influenced CSF production through hemodynamic changes or a direct action on the choroid plexus epithelium. Blood pressure data for carbachol,

phenylephrine, and salbutamol did not correlate with the changes in CSF production. Blood flow studies showed that none of the agonists caused statistically significant changes in cerebral or choroid plexus blood flow. These findings suggest that autonomic drugs probably act directly on the choroid plexus epithelium to change the rate of CSF formation.

Verapamil, an agent that blocks calcium channels, did not alter a carbachol-induced increase in fluid production indicating that the autonomic agents probably did not exert their effect by influencing calcium fluxes. The phosphodiesterase inhibitor, theophylline, on the other hand, increased the rate of CSF production. This effect suggested that the autonomic treatments might alter fluid formation by increasing or decreasing levels of the cyclic nucleotides guanosine 3',5' cyclic monophosphate (cGMP) or adenosine 3',5' cyclic monophosphate (cAMP).

Although the exact mechanisms remain to be elucidated, it is clear that cholinergic and $beta_2$ adrenergic receptors can be stimulated to increase CSF formation significantly and that the cholinergic pathway which predominates is also activated by alpha adrenergic agonists.

INTRODUCTION

Epithelia structurally similar to the choroid plexus epithelium are found in the ciliary body of the eye, the salivary gland, and the pancreas. Secretory transport in these tissues is known to be controlled to some extent by the autonomic nervous system. Therefore, it is of interest to consider the influence of the autonomic nervous system on the formation of cerebrospinal fluid (CSF).

The columnar epithelial cells of the choroid plexus are considered to be a primary site for the secretion of CSF. This structure is an evagination of the walls of the lateral and third ventricles of the brain and forms the roof of the fourth ventricle. The choroid plexus receives some innervation from the autonomic nervous system. On the ventricular side, the outermost structure of the choroid plexus is a single layer of epithelial cells supported by connective tissue richly supplied with blood vessels.

The autonomic nervous system is comprised of sympathetic and parasympathetic components. The sympathetic system has short fibers from the central nervous system to the sympathetic chain ganglia, then long

post-ganglionic fibers which innervate the tissues. Sympathetic effects are classified into alpha, $beta_1$, and $beta_2$ adrenergic actions and are mediated endogenously by the adrenergic neurotransmitters norepinephrine and epinephrine. Other agents can selectively stimulate alpha, $beta_1$, or $beta_2$ adrenergic activity if they have the necessary structural modifications required for each receptor. Phenylephrine is an alpha agonist; 1-isopropyl-amino-3(2 thiazoloxy)-2 propanol HCl (ITP) is a $beta_1$ agonist; and salbutamol is a $beta_2$ agonist. The parasympathetic system has long fibers from the central nervous system to ganglia close to the tissue being supplied, then short post-ganglionic fibers which innervate the tissue. The cholinergic neurotransmitter acetylcholine mediates parasympathetic actions. Chemically related agents such as carbachol can initiate cholinergic activity when applied to the effector site.

BACKGROUND

In all systems in which fluid formation can be altered by agents that affect the autonomic nervous system, one must consider two basic means by which these effects may be mediated. Alterations in fluid production may occur in direct response to autonomic agents acting on receptors located in the secretory cells. Also changes in fluid production may possibly result from changes in hemodynamic relationships increasing or decreasing pressure and flow in the vessels of the choroid plexus. Functionally, changes in hemodynamic relationships may interact with direct changes in the secretory function of the epithelium to alter fluid formation.

Autonomic Innervation and Function in Cerebral Blood Vessels

By following the nerve trunks passing into the brain, anatomists identifed nerves supplying the large pial vessels more than a century ago. Microscopists subsequently described the presence of nerve fibers in the finer cerebral vasculature, but classification of the nerves as sympathetic and parasympathetic did not come until the 1920's. Since the blood vessels of the choroid

plexus arise from cerebral arteries, a brief summary of general cerebrovascular innervation and autonomic function will be given before considering the choroid plexus specifically. A more detailed presentation of the autonomic innervation and responses of cerebral blood vessels is given in Appendix I.

Sympathetic innervation of the large pial blood vessels is supplied by the cervical sympathetic trunks. This has been demonstrated by showing the disappearance of adrenergic nerves after cervical sympathetic ganglionectomy. However, smaller parenchymal vessels within the brain receive adrenergic nerves from the brainstem. These nerves survive bilateral cervical sympathectomy. Parasympathetic innervation of cerebral blood vessels originates primarily through the facial nerve. Recent experiments have shown that cholinergic nerves also come from sources other than the cranial nerves.

A decrease in cerebral blood flow in dogs after electrical stimulation of the cervical sympathetic trunk or administration of norepinephrine has been demonstrated by some investigators; however, others have been unable to elicit these effects. On the other hand, stimulation of the facial nerve or exogenous administration of acetylcholine reproducibly and significantly increases cerebral blood flow.

Cerebral blood vessels have shown more consistent adrenergic responses *in vitro* than in the whole animal

experiments. Both electrical stimulation and alpha adrenergic agents contract cerebral arteries *in vitro*, but the maximum force of contraction is less than in arteries from other tissues. Beta adrenergic responses in cerebral vessels so far have been found only in the cat. In this species, $beta_2$ stimulation relaxes vessels contracted with serotonin.

The significant observation from both the *in vivo* and *in vitro* studies is that regardless of the nature of the stimulation (i.e., electrical or pharmacological) the adrenergic response of the cerebral vessel is small or absent. In contrast, cholinergic stimulation has a strong influence on cerebral blood vessels. *In vitro* acetylcholine has been shown to relax contracted vessels.

Innervation of the Choroid Plexus

Since the choroid plexus is composed of an epithelium resting on connective tissue containing blood vessels, it is not feasible to discuss separately innervation of the epithelium and innervation of the vessels with regard to what is presently known about the anatomy of the tissue. For this reason, evidence for nerves supplying both the epithelia and the blood vessels of this secretory tissue will be presented together.

Benedikt (1874) first observed nerve fibers around blood vessels and near epithelial cells of the fourth

ventricle plexus. These fibers arose from a vagal nucleus in the medulla which he called the XIII Cranial Nerve. Later, innervation in the lateral plexus was described as being mostly around the vessels, and it was suggested that these nerves might be involved in vasomotor function (Findlay, 1899). Stohr (1922) confirmed the observation of Benedikt in the fourth ventricle plexus and also demonstrated a nerve supply to the third ventricle plexus. He suggested the nerve fibers were divided into "vascular nerve fibers" entering with the arterial vessels and "choroid plexus proper" fibers originating in a vagal nucleus, in pontine nuclei, and in cerebral peduncles. Junet (1926) also described nerves supplying the epithelial cells of the plexus. Stohr's observation of separate vascular and epithelial innervation to the fourth ventricle plexus originating from the medulla was confirmed by Clark (1928, 1934), and he extended the theory to include the lateral plexus. Clark described the fibers as being mostly in the connective tissue base of the choroid plexus rather than around the epithelial cells. Some myelinated nerves and a large number of unmyelinated nerves were found in association with blood vessels and epithelial cells without any of them ending directly on capillaries. With electron microscopy, unmyelinated nerve fibers have been identified near the choroid plexus epithelium (Millen and Rogers, 1956) and around some blood vessels

supplying the plexus (Maxwell and Pease, 1956). Tennyson (1975) has described a nerve process adjacent to an epithelial cell. The cell membranes of both the nerve and epithelium were thickened, indicating the presence of a possible cell junction.

To determine the extent of adrenergic innervation, two groups of investigators did cervical sympathetic ganglionectomies. In the first study, a unilateral sympathectomy resulted in a patchy degeneration of nerve fibers in the connective tissue of the ipsilateral and third ventricle plexi of dogs and cats with most of the innervation remaining intact (Tsuker, 1947). The other study demonstrated by fluorescence microscopy the presence of an interconnected ground plexus of adrenergic nerves between the vascular walls and the epithelial cells (Edvinsson et al., 1974). These observations indicated the lateral and third ventricle plexi had the highest content of adrenergic innervation and the fourth ventricle plexus content was much lower. After a bilateral sympathectomy, the fluorescence disappeared in all the choroid plexus and the norepinephrine content of the plexus decreased significantly (Edvinsson et al., 1972b). Cholinergic innervation of the choroid plexus epithelium and blood vessels has also been described (Edvinsson et al., 1973b). In cats and rabbits, cholinergic fibers were observed for all the plexus in greater density than

adrenergic fibers. The network of cholinergic nerve fibers remained intact after sympathetic ganglionectomy.

Blood Flow to the Choroid Plexus

Blood flow to the choroid plexus has been measured in the intact whole animal in only one study. Alm and Bill (1973), using radioactive microspheres, obtained a control flow of 3.01 ml/gm min. Upon stimulation of the cervical sympathetic chain, the blood flow to the plexus did not change. Two groups have made *in situ* determinations of plexus blood flow. Welch (1963), while measuring CSF production at the lateral choroid plexus in the rabbit, observed a blood flow of approximately 2.86 ml/gm min. Pollay et al. (1972) perfused the choroid plexus vessels of sheep and related blood flow to the production of CSF. For a normal rate of formation they concluded choroid plexus blood flow must be about 2.56 ml/gm min. Pharmacological agents affecting blood flow were not used in any of these studies; however, Macri et al. (1966) and Politoff and Macri (1966) showed that serotonin and l-norepinephrine reduced choroidal blood flow in rabbits.

Autonomic Nervous System Control of Fluid Secretions

Fluid secretion occurs in several specialized epithelial tissues. Sympathetic or parasympathetic

agonists have been shown to influence secretion in nearly all of the secretory epithelia, but there has not been a consistent pattern of effects. The salivary gland is probably the most thoroughly studied of the tissues in which there is autonomic control over fluid production. In this system, both cholinergic and adrenergic agonists increase secretion (Koelle, 1970). Cholinergic agonists can produce approximately ten times as much fluid as beta adrenergic agonists (Mangos et al., 1973). Petersen (1972) has suggested that acetylcholine-activated secretion occurs as a result of an uptake of extracellular calcium which Douglas and Poisner (1963) showed was necessary for fluid movement. The calcium, in turn, may cause an efflux of potassium and an uptake of sodium which results in water transfer.

The eye is another site in which fluid movement is actively influenced by autonomic agonists. In this system both production of fluid by the ciliary body and drainage of aqueous humor through the trabecular meshwork are under autonomic control (Chiou and Zimmerman, 1975). It has been difficult to determine whether the cholinergic system or the adrenergic system really predominates in either mechanism, but it is certain that both are influential. Paterson and Paterson (1972) have shown that alpha adrenergic agonists cause a slight increase in aqueous humor production and outflow facility. In contrast,

beta$_2$ agonists (such as salbutamol) cause a decrease in production and a large increase in flow through the trabecular meshwork which can be blocked by propranolol (Langham and Diggs, 1974 and Langham, 1976). The effects of cholinergic agents on aqueous humor dynamics is more complicated. The major effect in the whole animal is an increased drainage of fluid (Ellis, 1971 and Macri and Cevario, 1973). When acetylcholine and eserine, an anti-cholinesterase agent, are given intra-arterially in an isolated perfused cat eye, the formation of aqueous humor increases 10 to 15 µl/min (Macri and Cevario, 1973). The action of acetylcholine can be blocked by atropine; however, it can also be prevented with alpha adrenergic receptor blocking agents or guanethidine, an adrenergic neuron blocking agent, indicating the effect is probably mediated by alpha receptors.

The acinar cells of the pancreas are also under autonomic control (Thomas, 1967). Either vagal stimulation or the administration of cholinomimetic drugs can elicit moderate increases in fluid secretion and substantial increases in the release of digestive enzymes. Norepinephrine and epinephrine decrease secretion possibly by constricting the blood vessels which supply the tissue.

Fluid transfer in the toad bladder and frog skin epithelia can be increased by beta adrenergic stimulation (Foster, 1974). This presumably occurs through changes

in adenosine 3',5' cyclic monophosphate (cAMP) levels since it can be mimicked by dibutyryl cAMP and potentiated by phosphodiesterase inhibitors (Jard, 1974). On the other hand, alpha adrenergic activation reduces water movement stimulated by theophylline or antidiuretic hormone (Foster, 1974 and Jard, 1974) but has no effect in the absence of these agents. No observations have been made concerning possible cholinergic actions.

Very little investigation has been made into the role of autonomic agents in the production of CSF. Becht and Gunnar (1921) set a needle in the cisterna magna of cats and noted that epinephrine and pilocarpine increased fluid outflow. They suggested that this was not true CSF production since some of the collected fluid was drawn up into the tube again after the action of the drug was finished. Bhattacharya and Feldberg (1958) observed the same effect during ventriculocisternal perfusion with epinephrine, histamine, and tubocurare. In these experiments only volumes were measured, and it was suggested that the increased flow during the perfusion of the ventricles was due to a change in cerebral blood flow. Vates et al. (1964) observed that norepinephrine (2.5×10^{-6} M in the perfusate) caused a decrease in production of about 24% during ventriculocisternal perfusion.

Rationale for Study

The evidence presented satisfies two major criteria for concluding that the autonomic nervous system may exert an influence on CSF production. First, it has been shown that the blood vessels and epithelia of the choroid plexus receive both adrenergic and cholinergic innervation. The adrenergic nerves extend from the cervical sympathetic trunk whereas the cholinergic nerves, comprising most of the tissue's innervation, originate from undefined locations. Second, it is clear that both adrenergic and cholinergic agents alter secretion in other epithelial systems.

Therefore, it seemed reasonable to investigate CSF production during pharmacological alteration of the adrenergic and cholinergic nervous system with agonists and antagonists and to examine the role of cerebral blood flow as a possible mediator of effects of these agents on CSF production. Such an investigation is the subject of this dissertation.

METHODS

Cats have been used extensively for studies of neurological and secretory functions in the brain and for this reason were chosen for investigation of the role of the autonomic nervous system on CSF production. Animals weighing 2-4 kg were anesthetized with an intrahepatic injection of 30 mg/kg sodium pentobarbital and further maintenance doses were given intravenously as needed throughout the experiment. Surgical procedures for the insertion of a tracheal cannula and femoral arterial and venous catheters were performed. Blood pressure and heart rate were monitored with a Statham pressure transducer connected to the femoral artery catheter and recorded on a Grass polygraph. Body temperature was maintained at 37° C. Throughout each experiment, the animal was artificially ventilated at a rate set to maintain arterial pH at approximately 7.35, the normal control value for a cat. By measuring arterial pH and adjusting the ventilatory rate as needed, the correct rate was established and the corresponding end-tidal CO_2 was recorded and subsequently monitored to maintain pH at the desired level.

For ventriculocisternal perfusion, the cat's head was placed in a standard stereotaxic apparatus. A midline

incision was made from the top of the head to the neck and a burr hole was drilled at a point 2 mm lateral to the saggital suture and 2 mm caudal to the coronal suture. A 22-gauge spinal needle, attached through a Statham pressure transducer to a Harvard infusion pump, was set in the right lateral ventricle. Placement was determined by a sharp drop in the pressure recording as the needle passed beyond the brain parenchyma into the ventricle. This needle was used to deliver perfusate and to monitor intraventricular pressure which was recorded throughout the experiment. The tip of a 19-gauge needle was then placed in the cisterna magna by separating the neck muscles at the midline and puncturing the dura mater just below the occipital condyle of the skull, and effluent was collected through a short tube attached to the needle. An artificial CSF (see Appendix II for the composition) was used for the perfusion. pH of the perfusion fluid was adjusted to 7.4 by bubbling 5% CO_2 in O_2 through it until the desired pH was achieved. The perfusion rate was 191 µl/min during the first 15 minutes and 76.4 µl/min thereafter. Following a 30 minute washout of the original CSF in the ventricular system, four to eight control periods of 15 minutes each were collected prior to tests with drugs or stimulation.

Production of CSF was calculated from the dilution of blue dextran, M.W. 2 X 10^6 daltons, (Pharmacia). The

collected effluent of each sampling period and the original artificial perfusate were diluted (100 µl in a total volume of 1.0 ml) for measurement of optical densities (Gilford 2400 spectrophotometer). This dilution was necessary to obtain a large enough volume to assay during short sampling periods. In most experiments, perfusion was continued six hours; none went beyond nine hours.

Fluid formation was calculated with the equation developed by Heisey et al. (1962) for dilution of non-diffusible substances:

$$V_f = \frac{(C_{in} - C_{out})(r)}{C_{out}}$$

The difference between the concentration of blue dextran in the infused buffer solution (C_{in}) and the effluent (C_{out}) divided by the concentration of the effluent is the dilution factor of the perfusate. The product of the dilution factor and the rate of infusion (r) then provides a value for the rate of formation of CSF.

Tests of Autonomic Nervous System Influence on CSF Production

Stimulation of Cervical Sympathetic Trunks

Bilateral electrical stimulation of the cervical sympathetic trunk was applied during ventriculocisternal perfusion in some experiments. In addition to the surgical procedure described for perfusion experiments, the cervical

sympathetic ganglion was exposed and the two preganglionic sympathetic fibers were isolated from the vagi. The preganglionic fibers were then passed through a platinum electrode which was connected to a stimulator. The nerve fibers were stimulated with 10 volts at a frequency of 10 shocks per second; each shock lasted for 0.5 msec. Nictitating membrane responses were also monitored during these experiments to insure that adequate stimulation was bilaterally applied. This was done by gently pulling each membrane away from the eye, placing a suture in it and attaching the suture to a Myograph-B (Narco Biosystems) transducer which permitted the recording of membrane responses. The experimental sampling period was 15 minutes in duration, with stimulation continuing throughout the period. Control samples were collected during two hours preceding and two hours following stimulation.

Modification of CSF Production by Application of Pharmacological Agents

Drugs tested to determine a possible autonomic influence on CSF production were carbamylcholine chloride (carbachol) (Sigma), phenylephrine HCl (Sigma), salbutamol (albuterol in the U.S.) (Schering), atropine methyl nitrate (Sigma), phentolamine mesylate (Ciba-Geigy), propranolol HCl (Ayerst), 1-isopropylamino-3(2 thiazoloxy)-2 propanol HCl (ITP) (Syntex), aminophylline (theophylline) (Parke-Davis), verapamil (Knoll Pharmaceutical), and methazolamide (Lederle). All autonomic agonists were

administered in volumes less than 1 ml; all other drugs were given in volumes less than 3 ml. These volumes were considered small enough not to alter significantly the total volume of blood.

At least one and one-half hours elapsed between administrations of autonomic agonists when the drugs were given to test the individual effects. No agonist was tested during the first 30 minutes after an autonomic blocking agent was given so as to test the effect of the blocking agent on CSF formation. Subsequently, agonists were injected at approximately 30 minute intervals.

The doses of the autonomic agonists used in these experiments were selected on the basis of the blood pressure dose-response curves. Doses on either side of the ED_{50} for blood pressure and the dose which gave the maximal blood pressure response were tested for their effect on CSF production. Salbutamol, however, required a dose higher than that giving maximal blood pressure effect to elicit a maximal effect on CSF formation. Doses of the autonomic antagonists used were chosen for their effectiveness in blocking peripheral actions of the agonists.

Cholinergic agonist and antagonist. Carbachol was chosen as the cholinergic agonist for its specificity at the cholinergic post-ganglionic effector site and prolonged half-life in the whole animal compared to acetylcholine.

It was dissolved in normal saline in concentrations up to 10 µg/ml and administered intravenously in doses of 0.03, 0.1, 0.3, or 3.0 µg/kg body weight. As in all experiments involving agonists, three 3-minute effluent samples were collected beginning immediately after injection of the drug.

Atropine (0.15 mg/kg) was given to test for a direct effect on CSF formation. During atropine treatment, carbachol was given in doses of 1.0, 3.0, and 30.0 µg/kg to test for blockade of cholinergic effects on blood pressure and CSF production. Phenylephrine (30 µg/kg) and salbutamol (3 µg/kg) were also administered during atropine treatment.

Alpha adrenergic agonist and antagonist. Phenylephrine was chosen as the agonist for these experiments because of its specificity for alpha adrenergic receptors. It was dissolved in normal saline in concentrations up to 300 µg/ml and administered intravenously in single doses of 3.0, 10.0, 30.0, and 100.0 µg/kg body weight or infused over a nine minute period (30-35 µg/kg min) intravenously. Single injections were followed by three 3-minute samples. During infusions 3-minute samples were also collected. Phentolamine was chosen as the alpha receptor-blocking agent for its specificity and reversibility. The direct action of phentolamine on CSF formation was tested at the dose of 2 mg/kg body weight. During

alpha adrenergic blockade, single injections of 30 µg/kg phenylephrine, 3 µg/kg salbutamol, and 3 µg/kg carbachol were tested. To further examine the mechanism of action of phenylephrine, another group of experiments was done in which the nine minute phenylephrine infusion was preceded three minutes earlier by an intravenous injection of 50 µg/kg of hemicholinium-3.

Beta adrenergic agonists and antagonist. To ascertain specificity of beta adrenergic function both a $beta_1$ and a $beta_2$ agonist were tested to determine their effect on CSF production. The $beta_1$ agent selected was 1-isopropylamino-3(2 thiazoloxy)-2 propanol HCl (ITP). This compound has been shown to be specific for cardio-acceleration and stimulation of lipolysis without affecting $beta_2$ actions (Lockwood and Lum, 1974). The drug was dissolved in normal saline less than five minutes before use to be certain that the drug was still active, and doses of 100 and 300 µg/kg body weight were administered intravenously. $Beta_2$ activity was determined with salbutamol. Salbutamol has been shown to have bronchodilator activity and to increase blood flow to the dog hindlimb more effectively than isoproterenol, and these effects are reduced in the presence of propranolol (Cullum et al., 1969). This drug was dissolved in normal saline in concentrations up to 200 µg/ml and was given intravenously in doses of 0.1, 1.0, 3.0, 10.0, and 30.0 µg/kg body weight. For both agents, cisternal effluent was collected

over three 3-minute periods after drug administration. The general beta blocking agent propranolol (2.0 mg/kg) was tested to determine its effect on CSF production and was given to antagonize $beta_2$ adrenergic effects. Phenylephrine and carbachol were also tested in the presence of propranolol.

Non-autonomic agents. Three non-autonomic drugs were studied with regard to their influence on CSF production: theophylline, verapamil, and methazolamide. Theophylline has been shown to stimulate the respiratory rate, increase urine flow, and stimulate the rate and force of contraction of the heart. Some of these actions of theophylline are thought to result from the ability of the agent to inhibit phosphodiesterase, the enzyme responsible for cyclic nucleotide hydrolysis (Amer and Kreighbaum, 1975). To test whether autonomic agents might be acting through changes in cyclic nucleotide levels to affect CSF production, theophylline was administered intravenously in the form of aminophylline in doses equivalent to 10 and 20 mg/kg theophylline. Again, CSF perfusate was collected over three 3-minute periods following drug injection. Verapamil has been demonstrated to block inward calcium channels in cardiac muscle (Kohlhardt et al., 1972). Since calcium influx is important in acetylcholine-stimulated salivary secretion, verapamil was given via the perfusate at a concentration of 10^{-5} M to test its

effect on carbachol activity; three 3-minute sampling periods followed the administration of carbachol. Carbonic anhydrase inhibitors, acetazolamide and benzolamide, have been shown to reduce CSF formation between 50 and 75% (Davson and Segal, 1970; Oppelt et al., 1964). The carbonic anhydrase inhibitor, methazolamide was chosen for its high lipid solubility providing ready access to the secretory tissues. In these experiments methazolamide (30 mg/kg) was given intravenously over a period of 30 minutes. CSF production in methazolamide treated cats was also studied in the presence of atropine (0.15 mg/kg) or phentolamine (2.0 mg/kg). Samples were collected in ten minute periods for at least two hours after both drugs had been administered.

Tests of Autonomic Nervous System Influence on Blood Flow

Radioactive microspheres were used to measure blood flow to the choroid plexus, brain, kidney, and biceps brachii muscle. The kidney and muscle flows were used as reference data. Briefly, the measurement depends on the immediate lodging of spheres in capillary beds proportional to the capillary flow of the tissue. After delivery of the spheres to the left side of the heart, the spheres will have the same distribution as the blood leaving the heart. Simultaneously withdrawing blood and microspheres

into a syringe at a known rate then provides the basis for estimation of flow in tissues (Wagner et al., 1969). Both femoral arteries and a femoral vein were catheterized, and a tracheal cannula was inserted. One of the arterial catheters was connected to a pump set to withdraw blood at 2.72 ml/min. The chest was opened at the midline and the pericardium incised. A catheter was placed in the left atrium and secured. The animal was then given 3500 units of sodium heparin to prevent clotting of blood. End-tidal CO_2 was carefully monitored during the experiment to insure the same pCO_2 during each injection of microspheres since brain blood flow is highly sensitive to pCO_2 changes.

The microspheres were suspended by the 3 M Company in 20% dextran in Tween 80® for injection. Microspheres 15 ± 5 microns in diameter were chosen because this size has been shown to be optimal for cerebral blood flow measurements, providing high reproducibility and less than 2% shunting of spheres past the capillary beds (Marcus et al., 1976). Buckberg et al. (1971) showed a minimum of 400 spheres must be present to measure reliably the blood flow of a tissue. Therefore, the number of counts per minute (cpm) per microsphere was determined to estimate a dose that would satisfy this requirement. In all experiments the cpm in each tissue represented more than the minimum required number of spheres, thus validating the calculation of flow from the radioactivity in the tissue. In each experiment injection of ^{85}Strontium spheres ten

seconds after beginning blood withdrawal gave control blood flows, and injection of ^{141}Cerium spheres later gave blood flows during the peak influence of 3 µg/kg carbachol, 30 µg/kg phenylephrine, or 3 µg/kg salbutamol on CSF production. The drugs were given about five seconds before the blood withdrawal began. Blood withdrawal was continued 90 seconds to guarantee at least one circulation time. The animal was sacrificed and the choroid plexus of the lateral and fourth ventricles was removed and weighed. The brain, kidney, and muscle were also taken, weighed, and ashed. The radioactivity was counted in a Beckman Biogamma Scintillation Counter.

Blood flow to each of the organs was calculated from the relation

$$\frac{\dot{Q}}{d} = \frac{F}{d'}$$

whence,

$$\dot{Q} = \frac{(F)(d)}{(d')}$$

where $\dot{Q}$ is the blood flow (ml/min), F is the rate at which the arterial blood is sampled, d is the cpm of the tissue being determined, and d' is the cpm of the isotope in the arterial blood sample. To standardize the values, the blood flows are expressed as ml/gm min considering tissue weight.

Evaluation of CSF Production Data

The changes in CSF production caused by the various treatments were calculated in the following manner: For agonists, a least squares linear regression was applied to the control CSF productions of each experiment. This regression was based on CSF formation during one or more control periods before any treatment and at least three post-treatment control periods as determined by the return of blood pressure and heart rate to pre-drug levels. No values for post-treatment controls were taken sooner than 30 minutes after administration of short-acting agonists such as carbachol, phenylephrine, and salbutamol. This line was used to determine the theoretical control CSF production at any given time over a two or three hour span of the experiment. The production rate measured over a three minute period after the agonist was given was compared with the theoretical control for that time, and the difference taken as the change in production caused by the drug. Since the autonomic blocking agents and methazolamide have relatively long active half-lives, control periods were not availble after administration of these drugs. For these experiments, four or more periods prior to the time the agent was given were used to determine a least squares regression line and calculate theoretical control CSF productions. Changes in production were determined in these experiments as for the agonists.

Statistical Analysis

All the changes in CSF production are expressed as mean ± standard error of the mean (SE). Statistical significance of changes in production caused by several treatments was established by pairing theoretical control and experimental data for each experiment and using the paired one-tailed t-test (Goldstein, 1964). Significance of changes in CSF production before and after treatment with blocking agents or methazolamide was determined by using the unpaired two-tailed t-test (Goldstein, 1964). To ascertain whether increasing doses of autonomic agonists yielded a dose-response relationship, the individual responses for each experiment were converted to probits, a measure of the per cent of maximum response obtained. A linear regression was then performed and a plot of log dose vs. probit was produced with a 95% confidence interval on the regression line. Significance of the line was established by testing its slope against a null hypothesis (Snedecor and Cochran, 1967). In all cases, significance was assumed when the t-value exceeded the minimum value for $p<.05$. Non-significance is denoted by N.S. in the data.

RESULTS

Ventriculocisternal Perfusion Studies for Estimation of CSF Production Rate

Control Experiments

Control data were collected in four experiments in which no drugs were given. Control data from 24 experiments in which only short-acting autonomic agonists such as carbachol, phenylephrine, and salbutamol were given were added to these, avoiding data collected within 30 minutes after drug. The two groups of data were combined since the slopes of the 4 untreated (-0.042 µl/min each minute) and 24 treated animals (-0.015 µl/min each minute) were not statistically significant from each other or different from zero when tested against a null hypothesis. These combined data, shown in Table 1 and Figure 1, indicate a steep increase in the optical density of the CSF perfusate reflected as a decrease in CSF production during the first 60 minutes of the experiment according to a regression line with a slope of -0.143 µl/min per minute. This initial apparent decline in fluid formation was probably due to an incomplete washout of the original CSF in the ventricular system. A more gradual decline follows ranging from 19.7 ± 1.0 to 15.8 ± 0.7 µl/min over

TABLE 1
CONTROL PRODUCTION OF CEREBROSPINAL FLUID

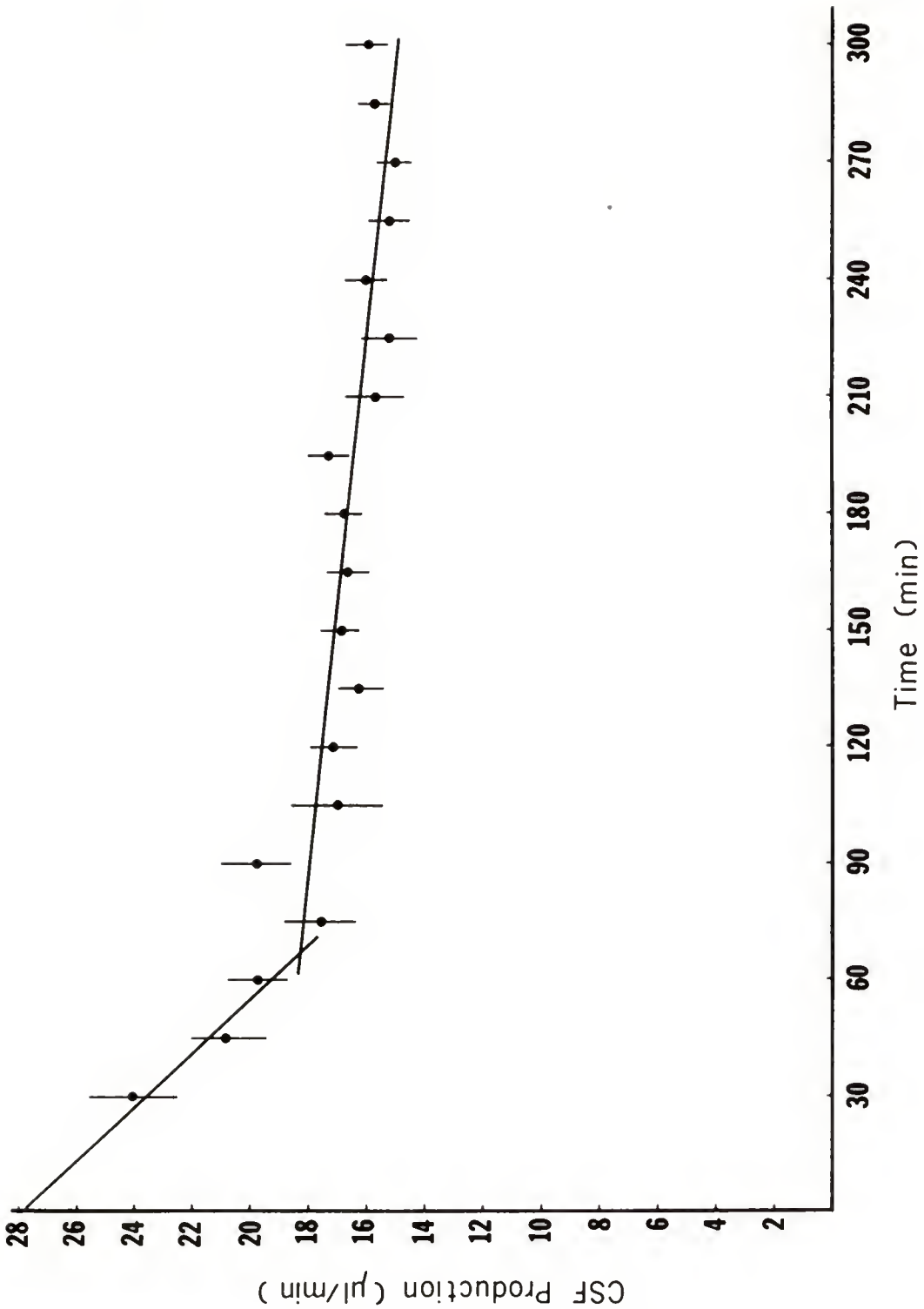
Time (min)[a]	n	Production (µl/min) ± SE
30	24	24.0 ± 1.5
45	21	20.7 ± 1.3
60	27	19.7 ± 1.0
75	10	17.5 ± 1.2
90	8	19.7 ± 1.2
105	6	16.9 ± 1.6
120	17	17.0 ± 0.8
135	22	16.2 ± 0.7
150	27	16.8 ± 0.7
165	24	16.5 ± 0.7
180	28	16.7 ± 0.6
195	11	17.2 ± 0.7
210	7	15.6 ± 1.0
225	14	15.1 ± 0.9
240	24	15.9 ± 0.7
255	20	15.1 ± 0.7
270	25	14.9 ± 0.6
285	22	15.6 ± 0.5
300	19	15.8 ± 0.7

Regression line for 0 to 60 minutes
$y = -0.143 x + 27.9$
$r = -0.96$

Regression line for 60 to 300 minutes
$y = -0.015 x + 19.3$
$r = -0.81$

[a]Duration of perfusion after washout of original CSF.

Figure 1. Changes in the production of CSF with time in control experiments. The ordinate is the mean CSF production value ± SE for at least 6 experiments at each point. The abcissa is time in minutes. The regression lines for 0 to 60 minutes and 60 to 300 minutes are represented by a solid line.


CSF Production (μl/min)
28
26
24
22
20
18
16
14
12
10
8
6
4
2
30
60
90
120
150
180
210
240
270
300
Time (min)

the next 240 minutes in which the slope of the regression line is -0.015 µl/min each minute. These data compare favorably with the accepted control CSF production value of approximately 20 µl/min (Davson, 1967) and with the slope of -0.025 µl/min per minute for a regression line on CSF formation in the monkey (Martins et al., 1974).

Cervical Sympathetic Stimulation

Only those experiments in which successful bilateral stimulation occurred were included in the study. This was determined by recording the contraction of the nictitating membrane. After an initial decrease in the response of the membrane during stimulation, the tension caused by the contraction remained steady, and no fatigue was observed during the experiment. Preganglionic stimulation of 10 volts at a frequency of 10 shocks/sec each with a duration of 0.5 msec decreased CSF production 3.0 ± 0.6 µl/min in five animals ($p<.05$ in paired t-test).

Cholinergic Agonist and Antagonist

Carbachol significantly increased the rate of CSF formation to 10.6 ± 2.1 µl/min over control (Table 2, Figure 2). Over a dosage range of 0.3 µg/kg to 3.0 µg/kg probit analysis (Figure 3) yielded a significant slope of 1.52 ($p<.05$). These relationships among the CSF production data were taken to indicate a carbachol dose-response. The ED_{50} for carbachol taken from the probit plot was 0.12 µg/kg. High doses of this cholinergic agonist occasionally produced slight increases in intra-ventricular pressure.

TABLE 2

CARBACHOL DOSE-RESPONSE: CHANGES IN CSF PRODUCTION

Dose (µg/kg)	Control CSF Production (µl/min) ± SE	Change in CSF Production[a] (µl/min) ± SE (n)
0.03	19.5 ± 2.2	+2.0 ± 0.6 (6)
0.10	28.9 ± 5.7	+4.6 ± 1.0 (5)
0.30	18.4 ± 1.1	+7.8 ± 1.6 (5)
3.0	16.8 ± 1.8	+10.6 ± 2.1 (6)
1.0[b]	15.8 ± 2.3	+0.6 ± 0.4 (3)
3.0[b]	14.1 ± 1.5	-0.3 ± 1.4 (3)[d]
30.0[b]	9.9 ± 2.1	+2.7 ± 1.0 (5)
3.0[c]	12.3 ± 0.6	+15.3 ± 6.5 (4)[e]

[a]Change in CSF production calculated for each experiment as the difference between the control and the peak effect of the drug

[b]Given within 120 min after 0.15 mg/kg atropine

[c]10^{-5}M verapamil in CSF perfusate

[d]$p < .05$ when compared with 3 µg/kg carbachol alone

[e]N.S. when compared with 3 µg/kg carbachol alone

Figure 2. Carbachol dose-response for CSF production. The ordinate is the mean increase in CSF production over the theoretical control value ± SE plotted on a linear scale. The abcissa is the dose of carbachol in µg/kg body weight plotted on a logarithmic scale. (●——————●) represents carbachol alone. (○) is carbachol during 0.15 mg/kg atropine.

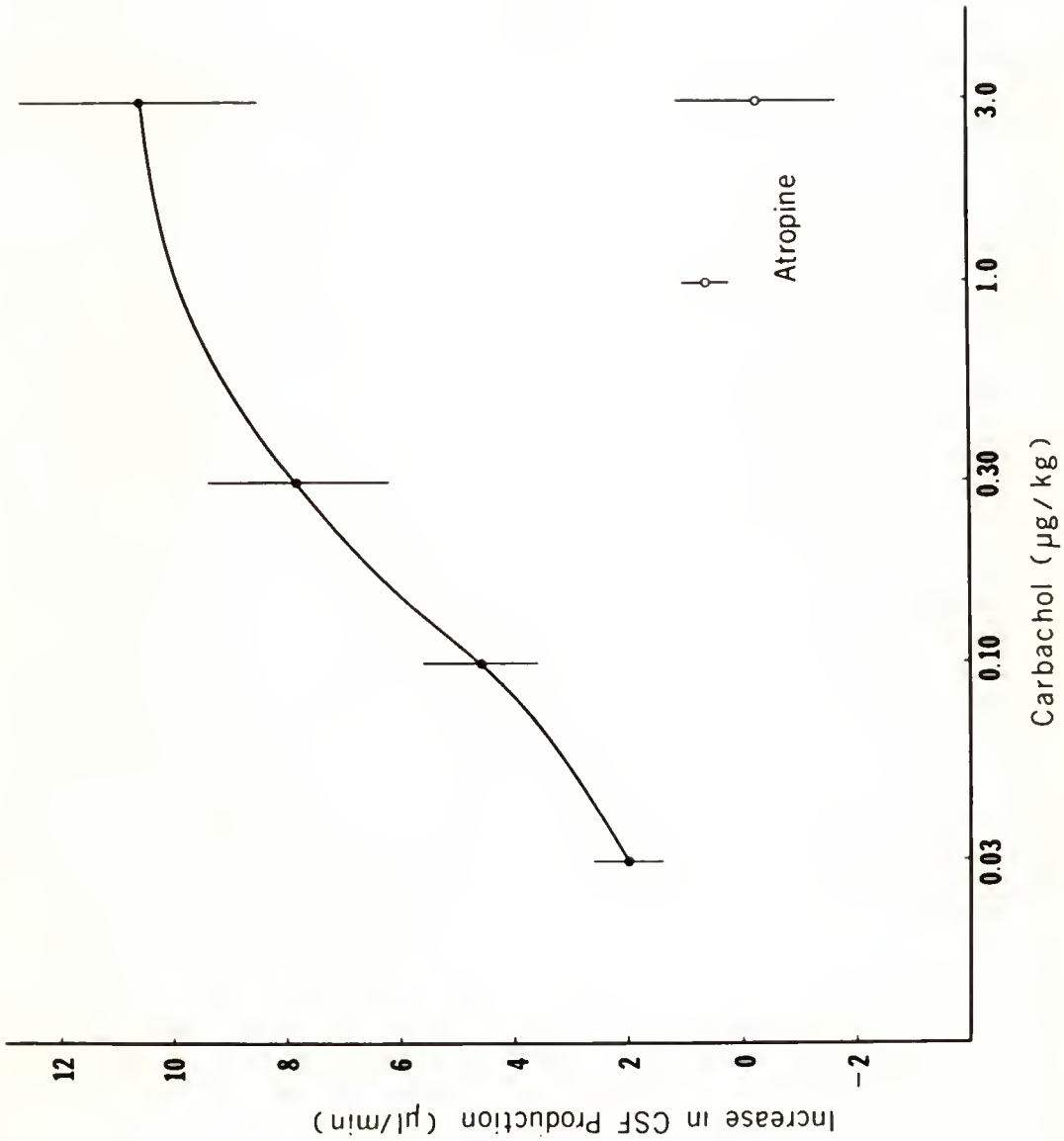
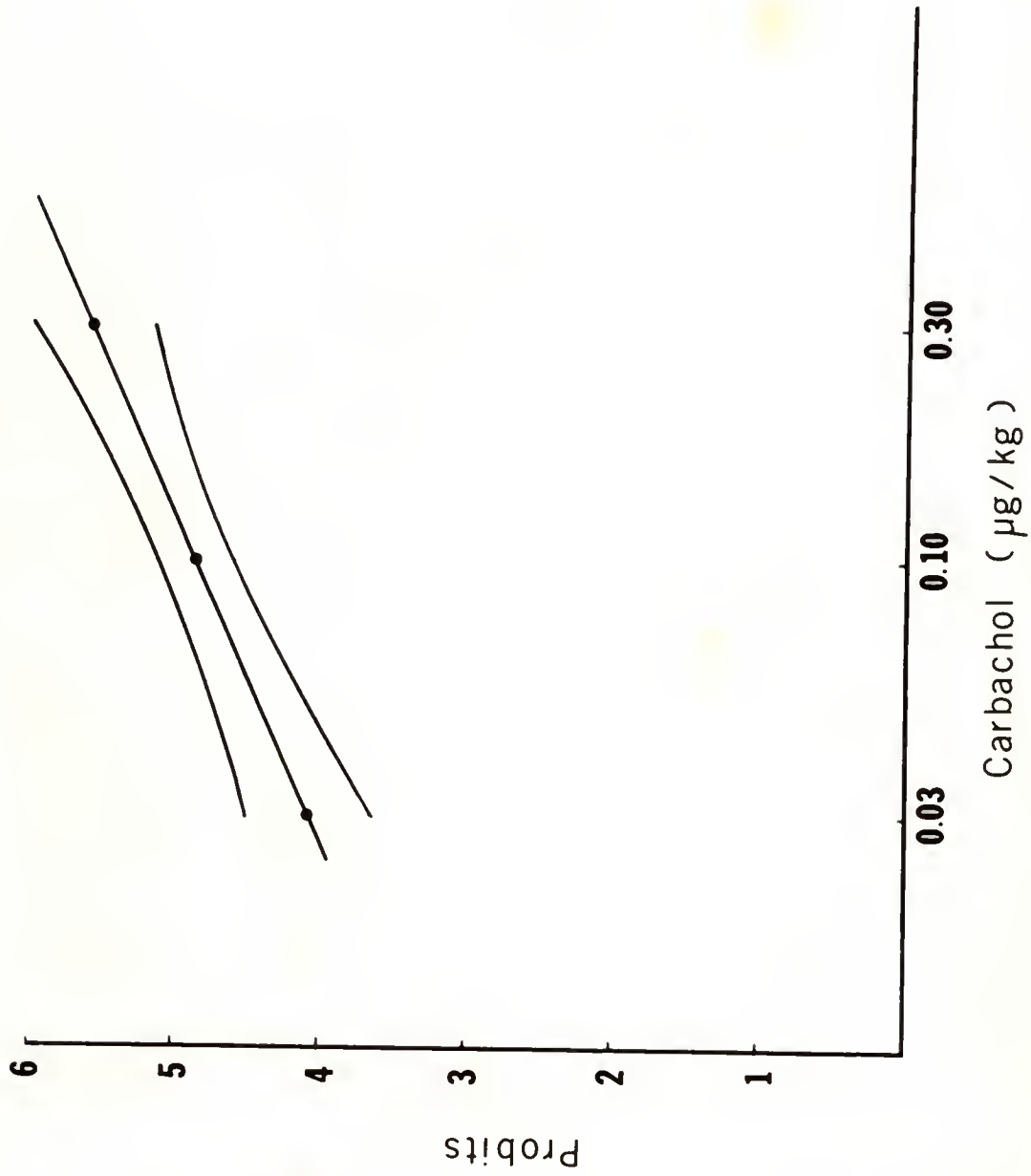

Increase in CSF Production (µl/min)
12
10
8
6
4
2
0
−2
0.03
0.10
0.30
1.0
3.0
Carbachol (µg/kg)
Atropine

Figure 3. 95% confidence interval on probit analysis of the effect of carbachol on CSF production. The ordinate is probits. The abcissa is the dose of carbachol in µg/kg body weight plotted on a logarithmic scale. The slope of the probit regression line is 1.52 which is significant ($p<.05$). The dose of carbachol producing an ED_{50} response is 0.12 µg/kg.


Probits
6
5
4
3
2
1
0.03
0.10
0.30
Carbachol (μg/kg)

Atropine caused a decrease of 2.6 ± 0.5 µl/min in CSF production which was statistically significant (Table 3). The cholinergic blocking agent also prevented the increase stimulated by 3 µg/kg carbachol (Table 2). The 30 µg/kg carbachol dose increased CSF production during atropine blockade by 2.7 ± 1.0 µl/min. The 3 µg/kg dose of carbachol was administered during phentolamine and propranolol blockade of adrenergic receptors in one or two experiments. No alteration in the effect of carbachol was evident. When verapamil (10^{-5} M) was present in the artificial CSF perfusate, there was also no change in the response to this dose of carbachol (Table 2). Verapamil alone did not alter normal CSF production.

Alpha Adrenergic Agonist and Antagonist

Phenylephrine caused a dose-dependent increase in CSF production (Table 4, Figure 4). The change in response with increasing amounts of drug was subjected to probit analysis (Figure 5). A slope of 3.41 was obtained from the plot indicating significance ($p<.05$). The ED_{50} for phenylephrine was 12 µg/kg. A slight increase in intraventricular pressure sometimes occurred with high doses of phenylephrine.

Phentolamine decreased production 3.7 ± 0.8 µl/min ($p<.05$) (Table 3). This alpha adrenergic blocking agent also significantly reduced the effect of 30 µg/kg phenylephrine, i.e., from 10.0 ± 1.6 µl/min to -0.8 ± 0.7 µl/min

TABLE 3

EFFECTS OF AUTONOMIC BLOCKING AGENTS ON CSF PRODUCTION

Drug	Dose (mg/kg)	Control CSF Production (µl/min) ± SE	Change in CSF Production[a] (µl/min) ± SE (n)
Atropine	0.15	17.1 ± 1.3	-2.6 ± 0.5 (9)[b]
Phentolamine	2.0	15.4 ± 1.0	-3.7 ± 0.8 (6)[b]
Propranolol	2.0	16.9 ± 1.8	-0.7 ± 0.6 (4)[c]

[a]Change in CSF production calculated for each experiment as the difference between the control and the peak effect of the drug

[b]$p < .05$ with paired t-test

[c]N.S. with paired t-test

TABLE 4

PHENYLEPHRINE DOSE-RESPONSE: CHANGES IN CSF PRODUCTION

Dose (µg/kg)	Control CSF Production (µl/min) ± SE	Change in CSF Production[a] (µl/min) ± SE (n)
3	16.2 ± 1.0	+0.3 ± 1.2 (5)
10	22.6 ± 3.9	+2.6 ± 0.6 (5)
30	20.0 ± 2.8	+10.0 ± 1.6 (7)
100	10.9 ± 2.0	+10.6 ± 1.5 (7)
30[b]	15.1 ± 1.2	-0.8 ± 0.7 (6)[d]
30[c]	15.8 ± 1.6	+0.4 ± 1.2 (5)[d]

[a]Change in CSF production calculated for each experiment as the difference between the control and the peak effect of the drug

[b]Given within 120 min after 2.0 mg/kg phentolamine

[c]Given within 120 min after 0.15 mg/kg atropine

[d]$p < .01$ when compared with 30 µg/kg phenylephrine alone

Figure 4. Phenylephrine dose-response for CSF production. The ordinate is the mean increase in CSF production over the theoretical control value ± SE plotted on a linear scale. The abcissa is the dose of phenylephrine in µg/kg body weight plotted on a logarithmic scale. (●——●) represents phenylephrine alone. (○) is 30 µg/kg phenylephrine during 2.0 mg/kg phentolamine. (▲) is 30 µg/kg phenylephrine during 0.15 mg/kg atropine.

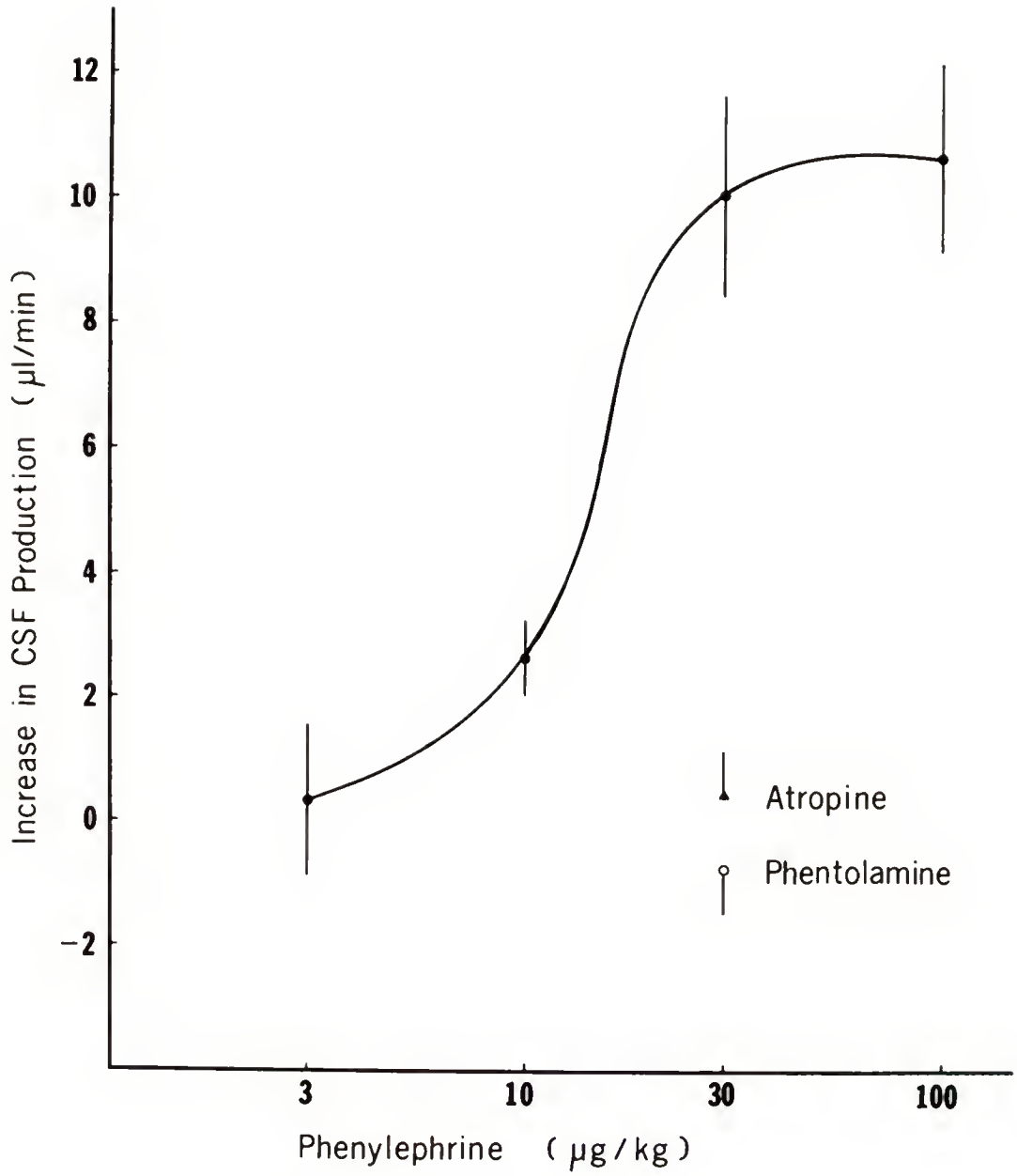
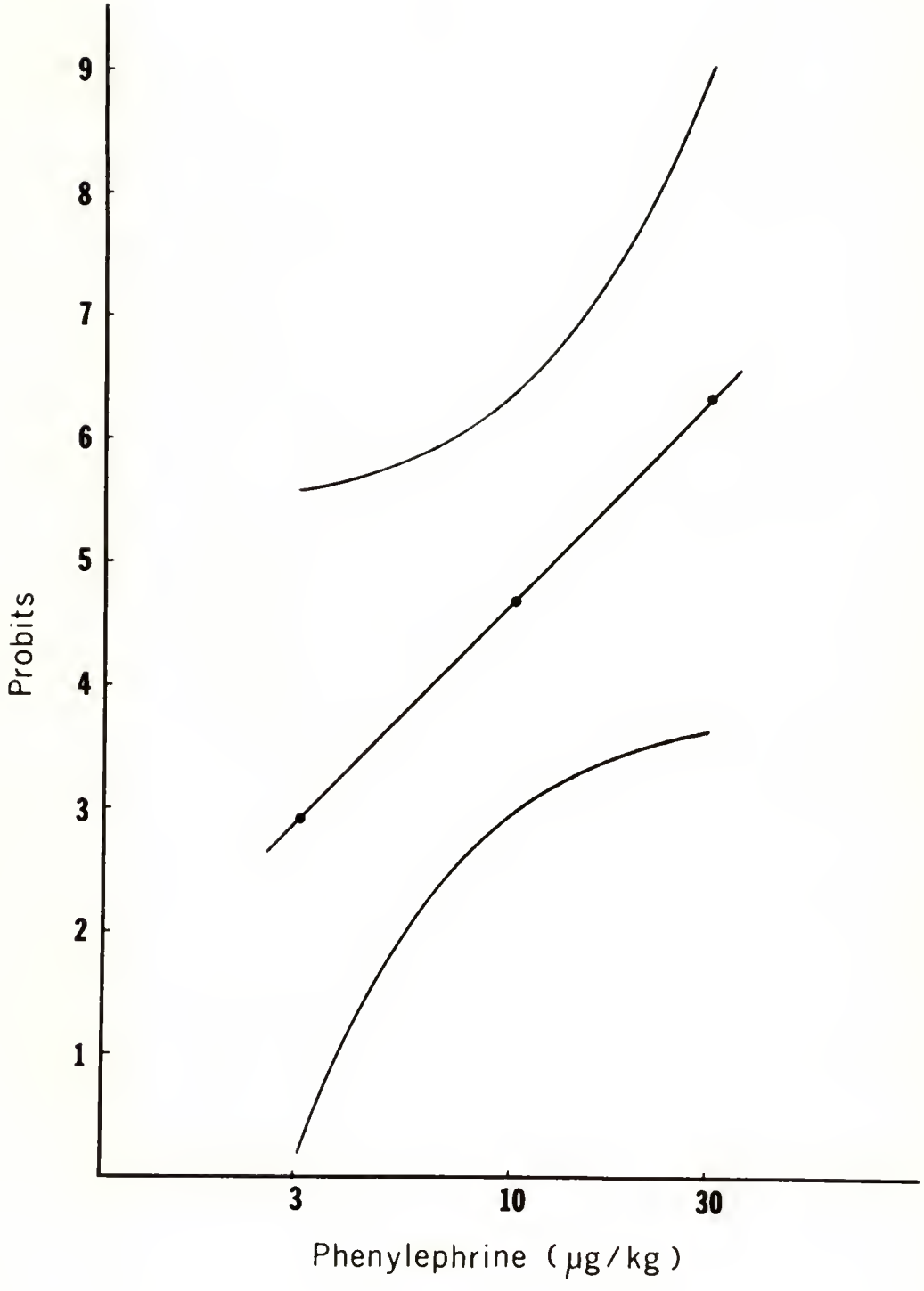

12
10
8
6
4
2
0
−2
Increase in CSF Production (μl/min)
3
10
30
100
Phenylephrine (μg/kg)
Atropine
Phentolamine

Figure 5. 95% confidence interval on probit analysis of the effect of phenylephrine on CSF production. The ordinate is probits. The abcissa is the dose of phenylephrine in µg/kg body weight plotted on a logarithmic scale. The slope of the probit regression line is 3.41 which is significant ($p<.05$). The dose of phenylephrine producing an ED_{50} response is 12 µg/kg.


9
8
7
6
5
4
3
2
1
Probits
3
10
30
Phenylephrine (μg/kg)

($p<.01$) (Table 4, Figure 4). Atropine, the cholinergic blocking agent, also effectively prevented the increase in CSF production caused by phenylephrine (Table 4, Figure 4). In the presence of atropine, 30 µg/kg phenylephrine increased CSF formation only 0.4 ± 1.2 µl/min which differed significantly ($p<.01$) from the increase due to the alpha agonist alone. Propranolol did not influence the effect of phenylephrine in the only experiment in which it was tested.

Phenylephrine was also administered in an intravenous infusion (approximately 30 µg/kg min) over nine minutes. In the first three minutes, CSF production increased 14.1 ± 4.0 µl/min, 24.1 ± 4.5 µl/min in the next three minutes, and 18.6 ± 2.0 µl/min in the final three minutes (Table 5). Administration of 50 µg/kg hemicholinium-3 to the animal three minutes prior to the phenylephrine infusion significantly reduced the increase in production in four of six cats. In these four animals, the increase in production due to phenylephrine was limited to 2.2 ± 1.0 µl/min ($p<.05$ compared with phenylephrine alone) in the first period, 2.5 ± 1.6 µl/min ($p<.01$) in the next three minutes, and 0.8 ± 1.8 µl/min ($p<.01$) in the final three minutes (Table 5). When the two non-responsive animals are included, the effect of hemicholinium pretreatment appears to cause no significant change in the effect of phenylephrine (Table 5). Hemicholinium alone did not influence the rate of CSF production.

TABLE 5

THE EFFECT OF HEMICHOLINIUM-3 (HC-3) ON CSF PRODUCTION DURING INTRAVENOUS INFUSION OF PHENYLEPHRINE (Phe)

	3 minutes	6 minutes	9 minutes
Change in CSF Production[a] (µl/min) ± SE during Phe[b] alone in four cats	+14.1 ± 4.0	+24.1 ± 4.5	+18.6 ± 2.0
Change in CSF Production (µl/min) ± SE during Phe after HC-3 pretreatment[c] in four cats	+2.2 ± 1.0[d]	+2.5 ± 1.6[e]	+0.8 ± 1.8[e]
Change in CSF Production (µl/min) ± SE during Phe after HC-3 pretreatment[c] in six cats	+7.0 ± 3.1[f]	+9.3 ± 4.4[f]	+8.0 ± 4.9[f]

[a] Change in CSF production calculated for each experiment as the difference between the control and the peak effect of the drug

[b] Phenylephrine infused intravenously for 9 minutes, approximately 30 µg/kg min

[c] Hemicholinium-3 (50 µg/kg) given 3 minutes prior to phenylephrine infusion

[d] $p < .05$ when compared with phenylephrine infusion alone

[e] $p < .01$ when compared with phenylephrine infusion alone

[f] N.S. when compared with phenylephrine infusion alone

Beta Adrenergic Agonists and Antagonist

The $beta_2$ agonist, salbutamol, increased CSF production about half as much as carbachol or phenylephrine (Table 6, Figure 6). A dose-response effect was demonstrated by a probit analysis of the changes in CSF formation (Figure 7). The regression line had a significant slope of 0.96 ($p<.05$). The ED_{50} for salbutamol was 1.5 µg/kg.

Beta blockade with propranolol did not change CSF production (Table 3). Propranolol did appear to reduce the increase observed with 3.0 µg/kg salbutamol, but the difference was not significant (Table 6, Figure 6). Atropine (four experiments) and phentolamine (one experiment) did not appear to alter the salbutamol response. The $beta_1$ agonist, ITP, did not significantly change the production of CSF in either 100 or 300 µg/kg doses.

Theophylline

Theophylline significantly increased CSF formation (Table 7). Doses of 10 and 20 mg/kg increased production 3.3 ± 0.8 µl/min and 9.2 ± 1.4 µl/min, respectively. The difference ($p<.01$) in the increases with the two doses suggest the effect was probably dose-dependent.

Carbonic Anhydrase Inhibition

The carbonic anhydrase inhibitor, methazolamide, significantly reduced the rate of CSF formation (Table 8). The maximal decrease was 11.2 ± 1.4 µl/min; over several

TABLE 6

BETA ADRENERGIC AGONISTS DOSE-RESPONSE: CHANGES IN CSF PRODUCTION

Dose (µg/kg)	Control CSF Production (µl/min) ± SE	Change in CSF Production[a] (µl/min) ± SE (n)
	SALBUTAMOL	
0.1	21.6 ± 1.8	+0.5 ± 0.5 (5)
1.0	22.0 ± 2.0	+3.3 ± 1.3 (4)
3.0	18.3 ± 1.8	+3.8 ± 1.6 (5)
10.0	17.4 ± 2.3	+4.0 ± 1.2 (7)
30.0	16.3 ± 1.0	+5.7 ± 1.4 (5)
3.0[b]	13.0 ± 2.7	+0.7 ± 0.2 (4)[e]
3.0[c]	10.1 ± 2.6	+2.8 ± 2.0 (4)[e]
	ITP[d]	
100	16.0 ± 1.1	-0.8 ± 1.0 (5)
300	19.5 ± 3.4	+0.1 ± 1.6 (4)

[a] Change in CSF production calculated for each experiment as the difference between the control and the peak effect of the drug

[b] Given within 120 min after 2.0 mg/kg propranolol

[c] Given within 120 min after 0.15 mg/kg atropine

[d] 1-isopropylamino-3(2 thiazoloxy)-2 propanol HCl

[e] N.S. when compared with 3.0 µg/kg salbutamol alone

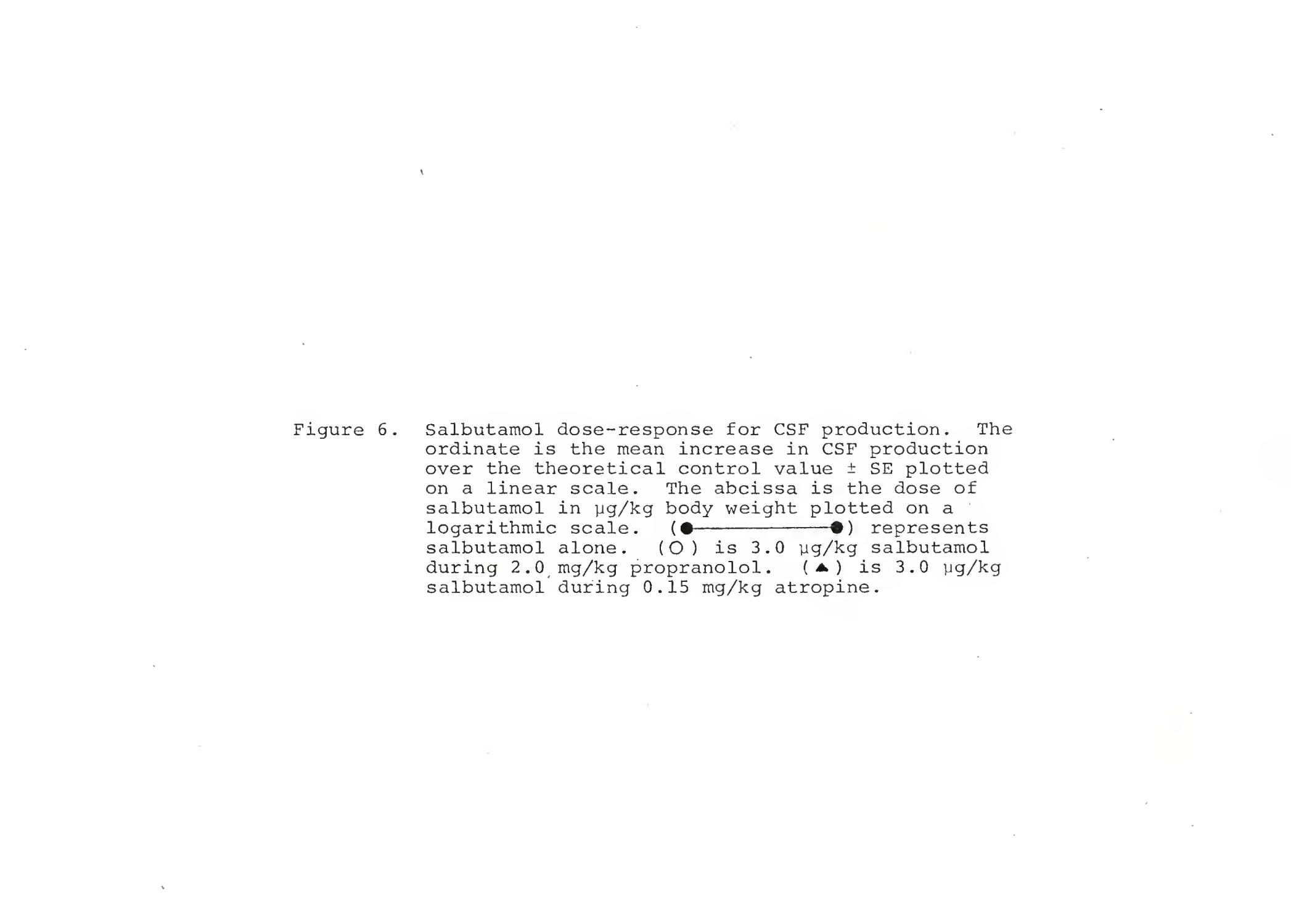


Figure 6. Salbutamol dose-response for CSF production. The ordinate is the mean increase in CSF production over the theoretical control value ± SE plotted on a linear scale. The abcissa is the dose of salbutamol in µg/kg body weight plotted on a logarithmic scale. (●——●) represents salbutamol alone. (○) is 3.0 µg/kg salbutamol during 2.0 mg/kg propranolol. (▲) is 3.0 µg/kg salbutamol during 0.15 mg/kg atropine.

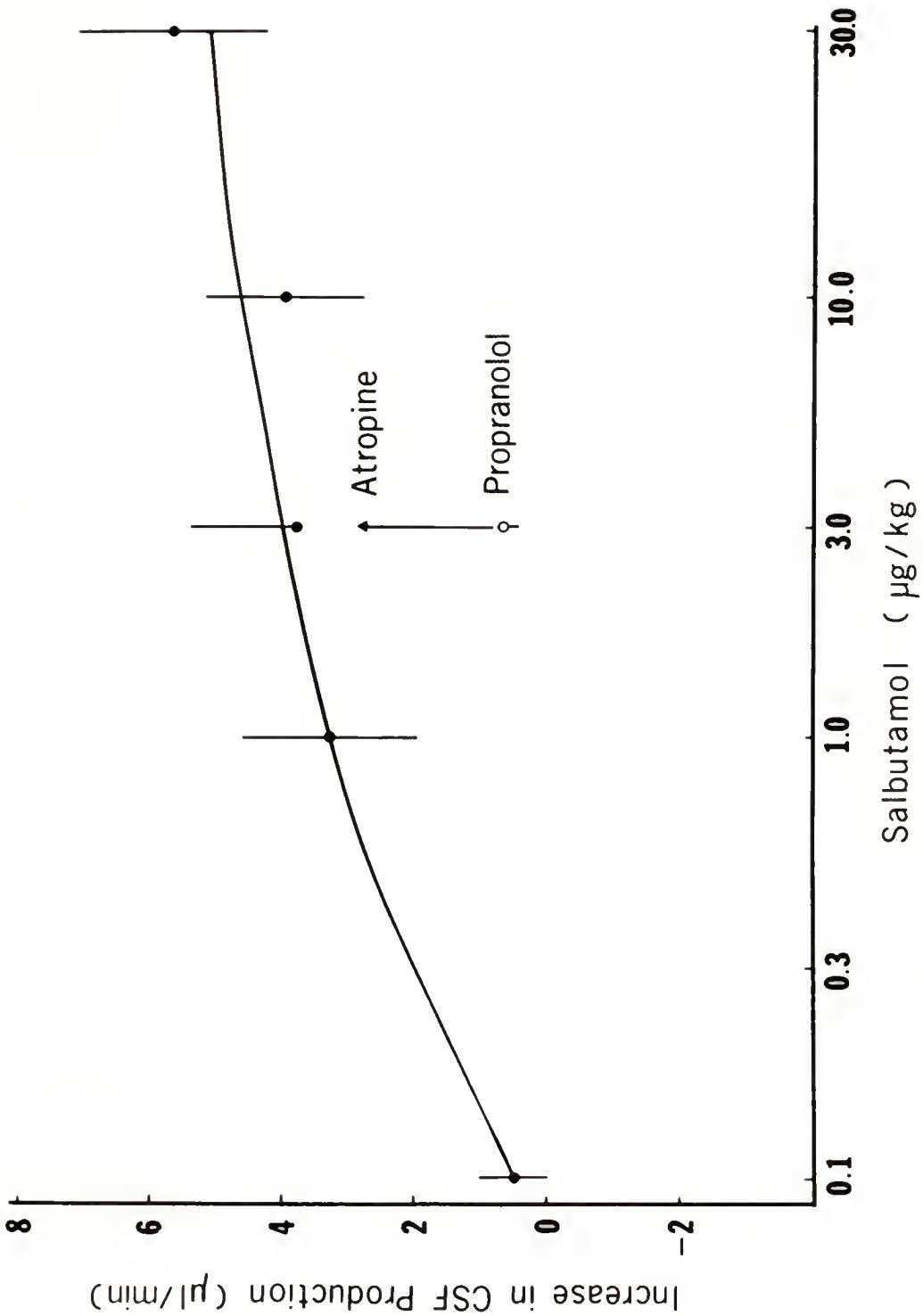

Increase in CSF Production (μl/min)
8
6
4
2
0
−2
0.1
0.3
1.0
3.0
10.0
30.0
Salbutamol (μg/kg)
Atropine
Propranolol

Figure 7. 95% confidence interval on probit analysis of the effect of salbutamol on CSF production. The ordinate is probits. The abcissa is the dose of salbutamol in µg/kg body weight plotted on a logarithmic scale. The slope of the probit regression line is 0.96 which is significant ($p<.05$). The dose of salbutamol producing an ED_{50} response is 1.5 µg/kg.

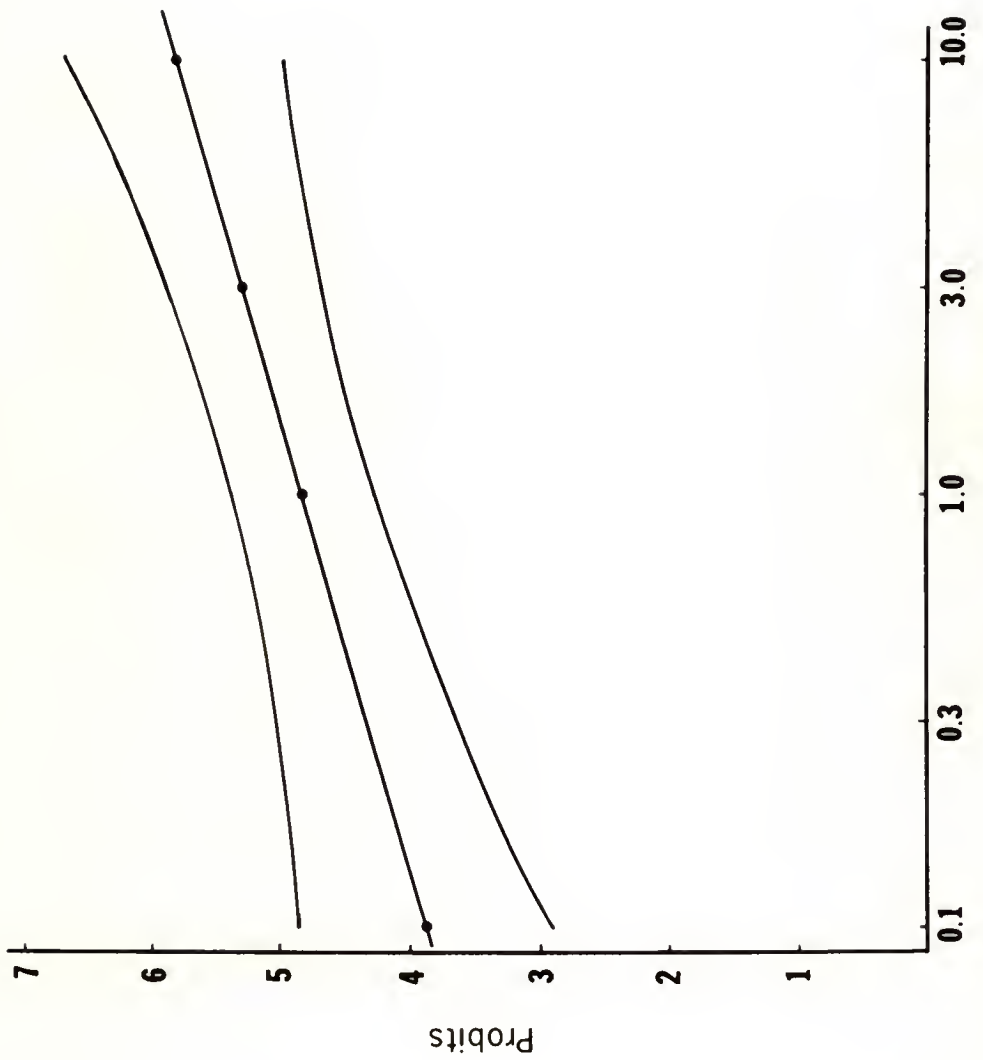

Probits
7
6
5
4
3
2
1
0.1
0.3
1.0
3.0
10.0
Salbutamol (µg/kg)

TABLE 7

EFFECTS OF THEOPHYLLINE ON CSF PRODUCTION, BLOOD PRESSURE, AND HEART RATE

Dose (mg/kg)	Control CSF Production (µl/min) ± SE	Change in CSF Production[a] (µl/min) ± SE (n)	Change in Mean Blood Pressure (mm Hg) ± SE (n)	Change in Heart Rate (beats/min) ± SE (n)
10	15.8 ± 2.1	+3.3 ± 0.8[b] (5)	-37 ± 6 (5)	+25 ± 5 (5)
20	16.9 ± 1.4	+9.2 ± 1.4[b,c] (4)	-54 ± 7 (4)	+35 ± 3 (4)

[a]Change in CSF production calculated for each experiment as the difference between the control and the peak effect of the drug

[b]$p < .01$ with paired t-test

[c]$p < .01$ when compared with 10 mg/kg theophylline

TABLE 8

EFFECTS OF CARBONIC ANHYDRASE INHIBITION ALONE AND WITH AUTONOMIC BLOCKING AGENTS ON CSF PRODUCTION

Treatment	Control CSF Production (µl/min) ± SE	Maximum Change in CSF Production[a] (µl/min) ± SE (n)	Mean Change in CSF Production[b] (µl/min) ± SE (n)
Methazolamide[c]	19.0 ± 1.7	-11.2 ± 1.4 (6)	-9.4 ± 1.2 (6)
Methazolamide + Phentolamine[d]	19.1 ± 2.4	-13.6 ± 1.8 (5)[f]	-12.5 ± 1.6 (5)[f]
Methazolamide + Atropine[e]	16.0 ± 2.2	-10.6 ± 0.9 (5)[f]	-8.8 ± 1.0 (5)[f]

[a] Calculated for each experiment as the difference between the control and the peak effect of the drug

[b] Calculated for each experiment as the difference between the control and the effect of the drug over two or more periods

[c] 30 mg/kg methazolamide given over 30 minutes

[d] 2.0 mg/kg phentolamine

[e] 0.15 mg/kg atropine

[f] N.S. when compared with methazolamide alone

collection periods the mean drop in production was 9.4 ± 1.2 µl/min. The CSF formation rate during methazolamide treatment was approximately 7 to 9 µl/min. Since atropine and phentolamine successfully decreased fluid formation, these drugs were tested in combination with methazolamide for possible additive effects. From Table 8 it is evident that the autonomic blocking agents caused no further significant decrease in production. It was also noted in several experiments that carbachol and phenylephrine partially override the carbonic anhydrase inhibition.

Changes in Blood Pressure and Heart Rate during Ventriculocisternal Perfusion Studies

Cervical Sympathetic Stimulation

Bilateral preganglionic cervical sympathetic stimulation (10 volts, 10 shocks/sec, each shock lasting 0.5 msec) produced an increase in mean arterial blood pressure of 42 ± 27 mm Hg. Heart rate did not change significantly.

Cholinergic Agonist and Antagonist

Doses of carbachol ranging from 0.03 to 3.0 µg/kg were administered. From Table 9 and Figure 8 it is clear that the drug had a dose-response effect in decreasing blood pressure ($p<.01$ by probit analysis). The heart rate also decreased beginning at the 3 µg/kg dose (Table 9). Atropine caused an initial drop of 43 ± 5 mm Hg in mean

TABLE 9

CARBACHOL DOSE-RESPONSE: CHANGES IN BLOOD PRESSURE AND HEART RATE

Dose (µg/kg)	Change in Mean Blood Pressure (mm Hg) ± SE (n)	Change in Heart Rate (beats/min) ± SE (n)
0.03	-28 ± 4 (7)	-1 ± 1 (7)
0.10	-38 ± 6 (6)	0 ± 2 (6)
0.30	-57 ± 5 (6)	3 ± 2 (6)
3.0	-81 ± 10 (6)	-63 ± 8 (5)
1.0[a]	-6 ± 3 (4)	-1 ± 3 (4)
3.0[a]	-9 ± 6 (2)[c]	0 ± 0 (2)[c]
30.0[a]	+12 ± 13 (5)	2 ± 2 (5)
3.0[b]	-50 ± 6 (4)[d]	-38 ± 15 (4)[d]

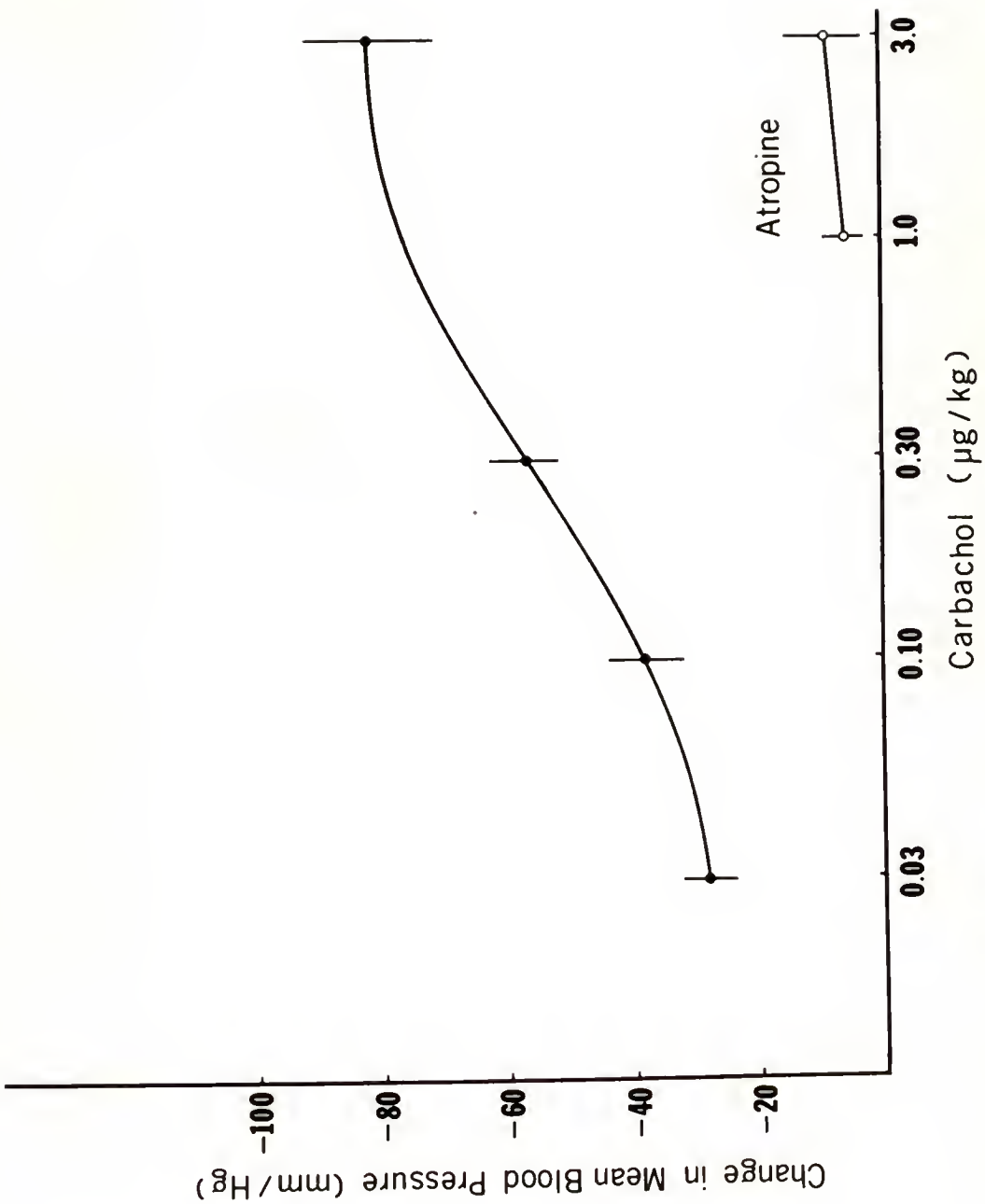
[a]Given within 120 min after 0.15 mg/kg atropine

[b]10^{-5}M verapamil in CSF perfusate

[c]$p < .01$ when compared with 3.0 µg/kg alone

[d]N.S. when compared with 3.0 µg/kg carbachol alone

Figure 8. Carbachol dose-response for blood pressure. The ordinate is the change in mean blood pressure (mm Hg) ± SE plotted on a linear scale. The abcissa is the dose of carbachol in µg/kg body weight plotted on a logarithmic scale. Carbachol alone is represented by (●——————●). Carbachol during 0.15 mg/kg atropine is represented by (○——————○).


Change in Mean Blood Pressure (mm/Hg)
-100
-80
-60
-40
-20
0.03
0.10
0.30
1.0
3.0
Carbachol (μg/kg)
Atropine

arterial blood pressure and a decrease in heart rate of 17 ± 3 beats/min (Table 10). Since both of these parameters had returned to pre-drug levels within 30 minutes after atropine administration, these actions were thought to be unrelated to the anti-cholinergic action of the drug. The atropine successfully blocked the fall in blood pressure seen with both 1 and 3 µg/kg carbachol and the decrease in heart rate seen with 3 µg/kg carbachol (Table 9). After atropine, 30 µg/kg carbachol produced a slight, but insignificant, increase in blood pressure, possibly through nicotinic stimulation of sympathetic ganglia. No change in heart rate was observed with this dose of carbachol during atropine treatment. The cardiovascular effects of 3 µg/kg were not affected by the presence of verapamil in the CSF perfusate (Table 9).

Alpha Adrenergic Agonist and Antagonist

Phenylephrine, given in doses between 3 and 100 µg/kg body weight, increased blood pressure to a peak of 133 ± 11 mm Hg (Table 11 and Figure 9). Probit analysis yielded a statistically significant slope ($p<.01$) and an ED_{50} of 17 µg/kg. Although the lower doses of phenylephrine (3 and 10 µg/kg) caused a significant decrease in heart rate, the larger amounts of drug caused no significant change (Table 11). Phentolamine caused a decrease in mean blood pressure of 40 ± 7 mm Hg and an increase in heart rate of 24 ± 5 beats/min (Table 10). Both blood pressure and heart rate returned to control values within 30 minutes.

TABLE 10

EFFECTS OF AUTONOMIC BLOCKING AGENTS ON BLOOD PRESSURE AND HEART RATE

Drug	Dose (mg/kg)	Change in Mean Blood Pressure[a] (mm Hg) ± SE (n)	Change in Heart Rate[a] (beats/min) ± SE (n)
Atropine	0.15	-43 ± 5 (17)	-17 ± 3 (16)
Phentolamine	2.0	-40 ± 7 (11)	+24 ± 5 (11)
Propranolol	2.0	-15 ± 2 (4)	-41 ± 11 (4)

[a]Measured within two minutes after injection

TABLE 11

PHENYLEPHRINE DOSE-RESPONSE: CHANGES IN BLOOD PRESSURE AND HEART RATE

Dose (μg/kg)	Change in Mean Blood Pressure (mm Hg) ± SE (n)	Change in Heart Rate (beats/min) ± SE (n)
3	+26 ± 4 (9)	-9 ± 4 (8)
10	+47 ± 6 (14)	-11 ± 3 (12)
30	+85 ± 9 (15)	+2 ± 5 (13)
100	+133 ± 11 (14)	-17 ± 12 (13)
30[a]	+21 ± 4 (6)[c]	-6 ± 4 (6)[d]
30[b]	+73 ± 5 (7)[d]	-13 ± 4 (7)[d]

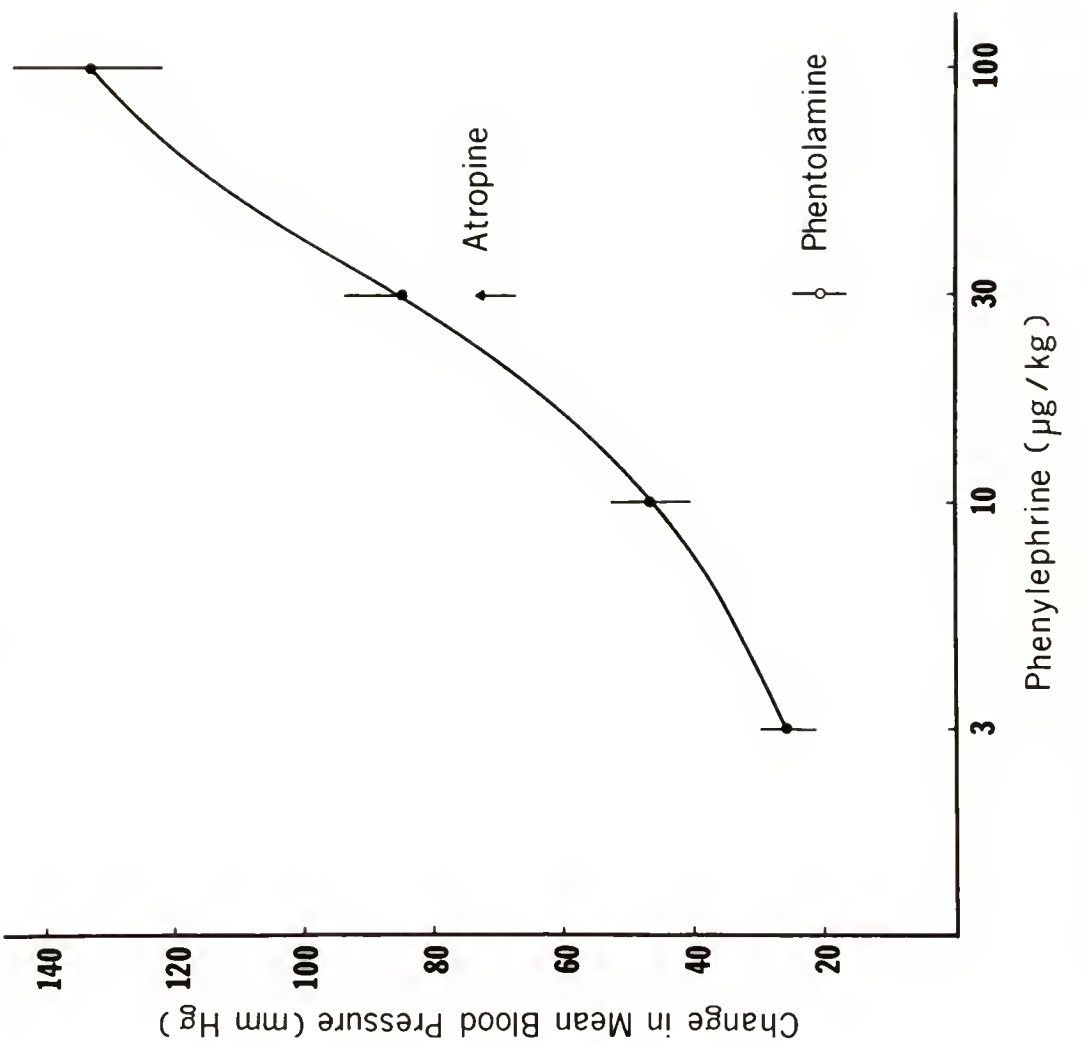
[a]Given within 120 min after 2.0 mg/kg phentolamine

[b]Given within 120 min after 0.15 mg/kg atropine

[c]$p < .05$ when compared with 30 μg/kg phenylephrine alone

[d]N.S. when compared with 30 μg/kg phenylephrine alone

Figure 9. Phenylephrine dose-response for blood pressure. The ordinate is the change in mean blood pressure (mm Hg) ± SE plotted on a linear scale. The abcissa is the dose of phenylephrine in µg/kg body weight plotted on a logarithmic scale. Phenylephrine alone is represented by (●——●). (○) is 30 µg/kg phenylephrine during 2.0 mg/kg phentolamine. (▲) is 30 µg/kg phenylephrine during 0.15 mg/kg atropine.


Change in Mean Blood Pressure (mm Hg)
140
120
100
80
60
40
20
Atropine
Phentolamine
3
10
30
100
Phenylephrine (μg/kg)

This blocking agent significantly reduced the pressor action of 30 µg/kg phenylephrine (Table 11). In the presence of atropine the phenylephrine-induced changes in blood pressure and heart rate were not altered (Table 11). Phenylephrine administered in an infusion (30 µg/kg min) over nine minutes increased mean arterial pressure by about 66 mm Hg (Table 12). Heart rate decreased significantly only in the first three minute period. Pretreatment with hemicholinium-3 did not significantly affect the increase in blood pressure or the change in heart rate due to phenylephrine (Table 12).

Beta Adrenergic Agonists and Antagonist

Salbutamol was given in doses between 0.1 and 30.0 µg/kg body weight. The blood pressure decreased significantly in response to all doses tested; however, the maximum fall occurred after 1.0 µg/kg (Table 13 and Figure 10). A dose-response increase in heart rate occurred as determined by probit analysis. Propranolol dropped the mean arterial blood pressure slightly and significantly decreased the heart rate immediately after administration (Table 10). Unlike the other autonomic blocking agents, these effects of propranolol persisted for two or more hours. Propranolol successfully blocked the cardiovascular responses to 3 µg/kg salbutamol (Table 13). Atropine did not significantly alter the changes in mean blood pressure and heart rate caused by

TABLE 12

THE EFFECT OF HEMICHOLINIUM-3 (HC-3) ON BLOOD PRESSURE AND HEART RATE DURING INTRAVENOUS INFUSION OF PHENYLEPHRINE (Phe)

	3 minutes		6 minutes		9 minutes	
	Phe[a]	HC-3 + Phe[b]	Phe	HC-3 + Phe	Phe	HC-3 + Phe
Change in Mean Blood Pressure (mm Hg) ± SE (n)	+69 ± 3 (4)	+63 ± 10[c] (6)	+70 ± 6 (4)	+69 ± 9[c] (6)	+60 ± 7 (4)	+63 ± 11[c] (6)
Change in Heart Rate (beats/min) ± SE (n)	-32 ± 13 (4)	-30 ± 15[c] (6)	+6 ± 23 (4)	-16 ± 19[c] (6)	-10 ± 19 (4)	-7 ± 20[c] (6)

[a]Phenylephrine infused intravenously for 9 minutes, approximately 30 µg/kg min

[b]Hemicholinium-3 (50 µg/kg) given 3 minutes prior to phenylephrine infusion

[c]N.S. when compared with phenylephrine infusion alone

TABLE 13

BETA ADRENERGIC AGONISTS DOSE-RESPONSE: CHANGES IN BLOOD PRESSURE AND HEART RATE

Dose (µg/kg)	Change in Mean Blood Pressure (mm Hg) ± SE (n)	Change in Heart Rate (beats/min) ± SE (n)
	SALBUTAMOL	
0.1	-27 ± 5 (6)	+3 ± 3 (6)
1.0	-40 ± 8 (9)	+17 ± 4 (8)
3.0	-43 ± 7 (9)	+33 ± 5 (9)
10.0	-45 ± 5 (9)	+39 ± 6 (9)
30.0	-45 ± 5 (7)	+58 ± 8 (7)
3.0[a]	-10 ± 2 (4)[d]	+1 ± 1 (4)[d]
3.0[b]	-19 ± 13 (4)[e]	+18 ± 5 (4)[e]
	ITP[c]	
100	-11 ± 6 (5)	+21 ± 9 (5)
300	-17 ± 5 (4)	+20 ±11 (4)

[a]Given within 120 min after 2.0 mg/kg propranolol

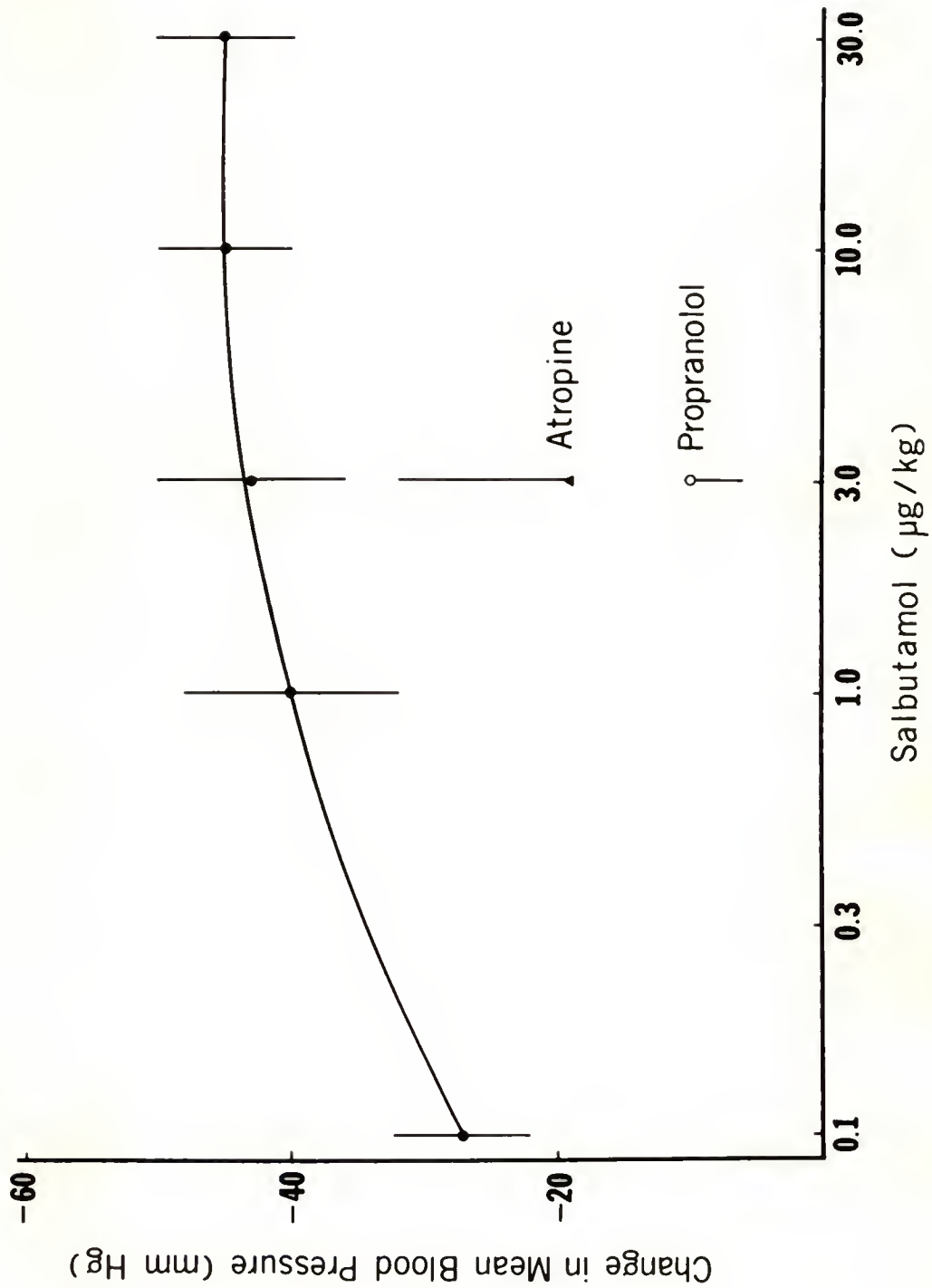
[b]Given within 120 min after 0.15 mg/kg atropine

[c]1-isopropylamino-3(2 thiazoloxy)-2 propanol HCl

[d]$p < .05$ when compared with 3.0 µg/kg salbutamol alone

[e]N.S. when compared with salbutamol alone

Figure 10. Salbutamol dose-response for blood pressure. The ordinate is the change in mean blood pressure (mm Hg) ± SE plotted on a linear scale. The abcissa is the dose of salbutamol in µg/kg body weight plotted on a logarithmic scale. Salbutamol alone is represented by (●——●). (○) is 3.0 µg/kg salbutamol during 2.0 mg/kg propranolol. (▲) is 3.0 µg/kg salbutamol during 0.15 mg/kg atropine.


Change in Mean Blood Pressure (mm Hg)
-60
-40
-20
0.1
0.3
1.0
3.0
10.0
30.0
Salbutamol (μg/kg)
Atropine
Propranolol

salbutamol. The $beta_1$ agonist, ITP, increased heart rate at 100 and 300 µg/kg doses (Table 13). A small decrease in mean arterial blood pressure was also observed.

Non-autonomic Drugs

Theophylline given intravenously significantly decreased mean arterial blood pressure and increased the heart rate (Table 7). In both cases the effects appeared to be dose-related. The dose of 10 mg/kg decreased blood pressure 37 ± 6 mm Hg and increased heart rate 25 ± 5 beats/min, whereas 20 mg/kg decreased blood pressure 54 ± 7 mm Hg and increased heart rate 35 ± 3 beats/min. Verapamil in the perfusate did not influence peripheral blood pressure or heart rate. Methazolamide also had no significant peripheral cardiovascular effects.

Effect of Autonomic Agonists on Blood Flow

One group of three cats served as an internal control; i.e., each animal received two control injections of microspheres sequentially. In these animals, no changes in blood flow were detected in any of the four tissues (Table 14). Other groups of cats received 30 µg/kg phenylephrine, 3.0 µg/kg carbachol, or 3.0 µg/kg salbutamol as the experimental treatment during the second injection of microspheres. The control blood flows (ml/gm min) for these animals were 2.26 ± .28 for the

TABLE 14

EFFECT OF PHENYLEPHRINE, CARBACHOL, AND SALBUTAMOL ON BLOOD FLOW TO THE CHOROID PLEXI, BRAIN, SKELETAL MUSCLE, AND KIDNEY

Treatment		Choroid Plexi		Brain		Skeletal Muscle		Kidney	
		Control Flow[a]	Change After Treatment	Control Flow	Change After Treatment	Control Flow	Change After Treatment	Control Flow	Change After Treatment
Control	$\bar{x}$	1.94	-.06	.42	+.02	.028	-.001	1.63	-.14
	SE	.46	.18	.07	.06	.006	.004	.37	.19
Carbachol 3 µg/kg	$\bar{x}$	2.33	+.81	.37	-.06	.041	+.018	1.94	-.58
	SE	.79	.93	.02	.04	.012	.011	.23	.11
Phenylephrine 30 µg/kg	$\bar{x}$	2.51	+.77	.63	-.09	.070	+.013	1.24	-.58
	SE	.70	.86	.09	.16	.025	.019	.43	.36
Salbutamol 3 µg/kg	$\bar{x}$	2.16	+.35	.40	+.01	.049	+.024	1.74	-.45
	SE	.33	.50	.09	.05	.011	.028	.49	.26
Mean Control Flow	$\bar{x}$	2.26		.46		.048		1.64	
	SE	.28		.04		.008		.19	

[a]Blood flow expressed as ml/gm min wt tissue weight

choroid plexus, 0.46 ± .04 for the brain, .048 ± .008 for the muscle, and 1.64 ± .19 for the kidney. None of the drugs caused a significant change in blood flow to any of the tissues examined (Table 14).

DISCUSSION

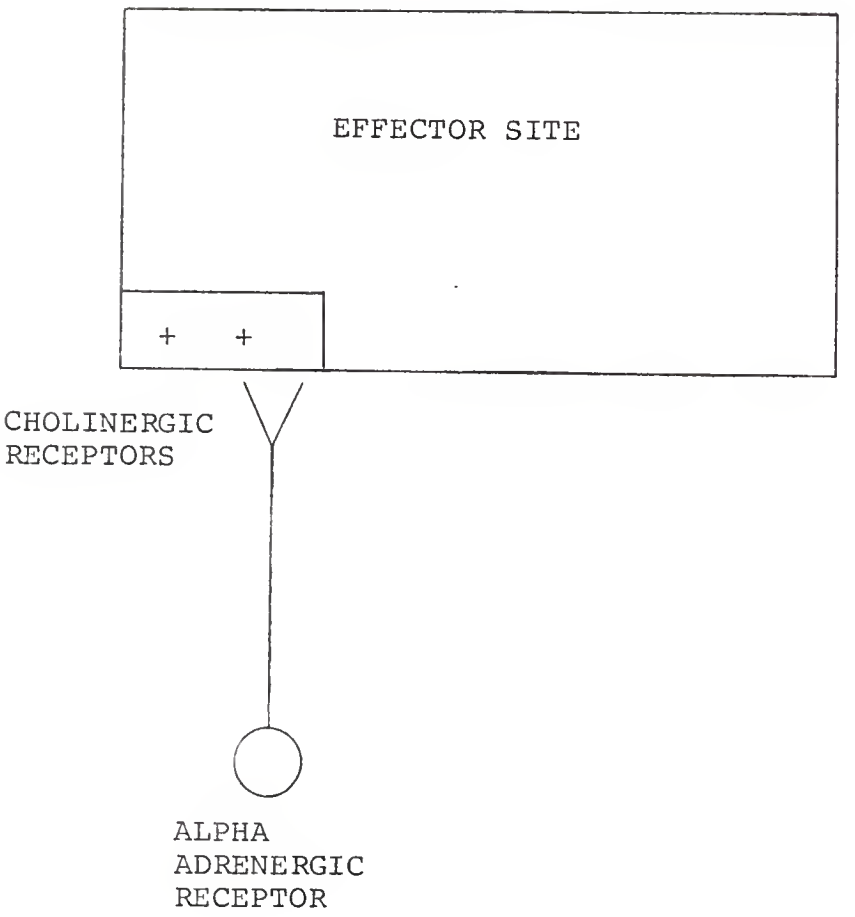
Modification of CSF Production by Autonomic Influences

The data show significant increases in CSF production as a result of stimulation of the cholinergic system by pharmacological agents that influence the autonomic nervous system. The cholinergic agonist, carbachol, caused a dose-dependent increase in the rate of CSF formation up to 10.6 ± 2.1 µl/min that was blocked by atropine. Further evidence for cholinergic control of secretion is found in the phenylephrine data. This alpha adrenergic agonist also increased CSF formation by 10.6 ± 1.5 µl/min; this increase was blocked by both phentolamine and atropine. These data suggested that the alpha adrenergic agonist probably was stimulating a cholinergic pathway to release acetylcholine to increase CSF production. Since hemicholinium blocks the uptake of choline for the synthesis of acetylcholine (Gardiner, 1957), hemicholinium was given prior to an infusion of phenylephrine. The infusion of phenylephrine without hemicholinium increased the production rate to more than twice that following a single injection of the drug. However, when the animal was pretreated with the hemicholinium, the infusion of phenylephrine was not able to increase fluid production

in four of six animals. These experiments confirmed that the increase in CSF formation caused by phenylephrine depends on the synthesis and release of acetylcholine. This relationship is depicted in Figure 11 which shows alpha adrenergic receptors that may or may not be supplied by a neuron. The failure of hemicholinium in the other two animals might be explained by larger stores of acetylcholine or particularly high levels of choline. An inadequate concentration of hemicholinium is probably not a cause for this lack of effect.

A marked similarity exists among several secretory systems with respect to cholinergic function and parasympathetic innervation. Cholinergic stimulation of the salivary glands causes a profuse secretion (Koelle, 1970). Parasympathetic innervation for these glands originates from the inferior and superior salivary nuclei of the brainstem (Mitchell, 1953). Secretion by the pancreas and by the stomach can also be enhanced by cholinergic agonists (Koelle, 1970). Both of these tissues derive their parasympathetic innervation from the dorsal vagal nucleus which is located immediately adjacent to the salivary nuclei in the brainstem (Mitchell, 1953). The production of aqueous humor by the ciliary body is also stimulated by cholinergic agents (Macri, 1971). The source of the cholinergic innervation in this tissue is not defined. Increase in aqueous humor formation is

Figure 11. Autonomic pathways to the choroid plexus I. The effector site has a cholinergic receptor. (++) indicates the relative effectiveness of the activated receptor to increase CSF production. The cholinergic nerve supplying the choroid plexus, in turn, can be stimulated by alpha adrenergic agonists. The location of the alpha adrenergic receptor remains undefined.


EFFECTOR SITE
+ +
CHOLINERGIC
RECEPTORS
ALPHA
ADRENERGIC
RECEPTOR

apparently mediated by a preganglionic cholinergic stimulation of an adrenergic effector (Macri, 1971).

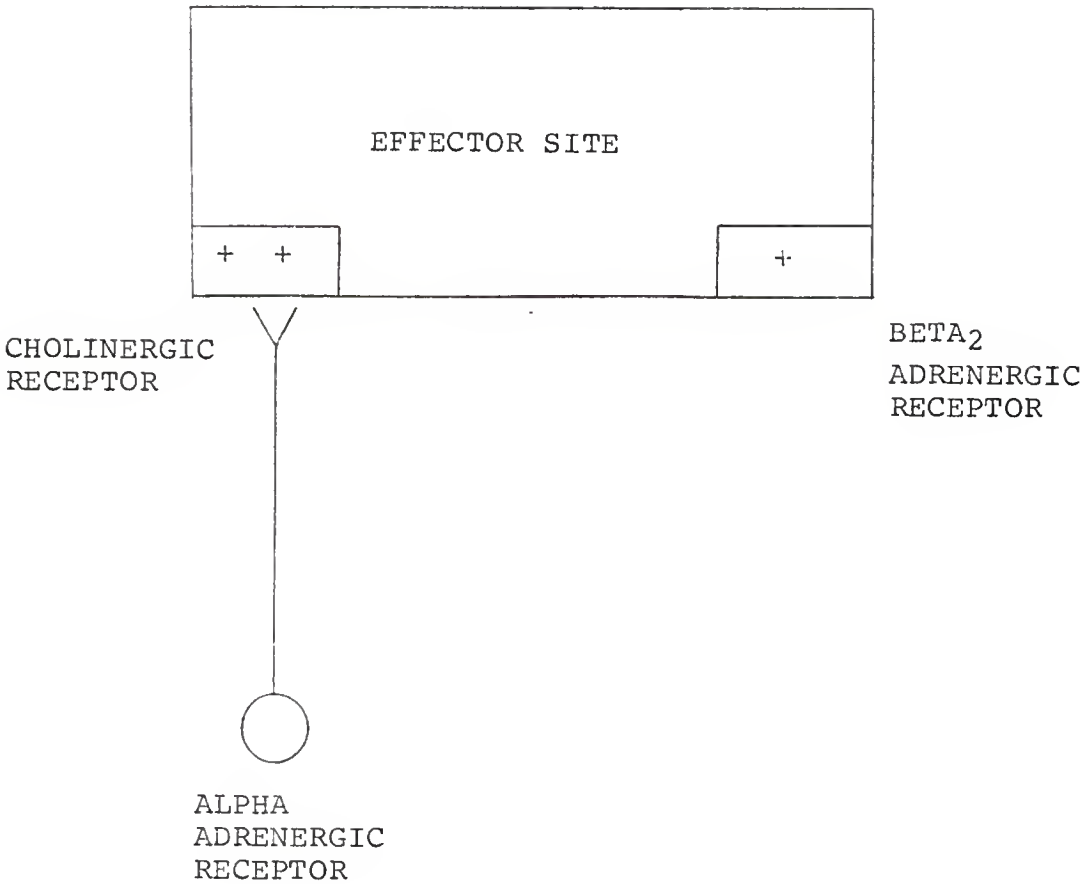
The origin of the cholinergic nerve supply to the choroid plexus is unknown. Early anatomical descriptions of nerves in the choroid plexus suggested that the innervation might originate in the dorsal vagal nucleus of the brainstem (Benedikt, 1874; Stohr, 1922; Clark, 1932). Although there are no data confirming that these are cholinergic nerves, they arise in the same area responsible for parasympathetic-stimulated pancreatic and gastric secretion. This area is immediately adjacent to the superior and inferior salivary nuclei (Mitchell, 1953). In spite of the proximity of origin to other secretory nuclei, one major observation thus far marks the cholinergic-stimulated production of CSF as unique among cholinergic secretory systems: the activation of cholinergic pathways by alpha adrenergic agonists.

As in most secretory systems, for the choroid plexus it is unknown whether the cholinergic effector site is vascular or epithelial. Cerebral blood vessels receive some cholinergic innervation from the facial, glossopharyngeal, and vagal cranial nerves (Mitchell, 1953) and from pathways arising within the brain (Edvinsson, 1975). Innervation from within the central nervous system has also been shown for the blood vessels and the epithelium of the choroid plexus (Clark, 1928 and 1932).

The role of the sympathetic nervous system in the production of CSF is complex. The action of the alpha adrenergic agonist, phenylephrine, in stimulating fluid formation through a cholinergic pathway (Figure 11) has already been discussed. The $beta_2$ agonist, salbutamol, also caused a dose-dependent increase in CSF formation; however, the maximum effect was an increment of 5.7 ± 1.4 µl/min which is approximately half of the cholinergic response. The action of salbutamol appeared to result from a direct stimulation of beta receptors at the effector site (Figure 12) since neither atropine nor phentolamine influenced its effect, whereas the general beta blocking agent, propranolol, did reduce the salbutamol effect. $Beta_1$ receptors are apparently absent in the choroid plexus, for ITP did not influence production at all.

Since norepinephrine is present in the choroid plexus and disappears after cervical sympathectomy (Edvinsson et al., 1972b; Edvinsson et al., 1974), alpha adrenergic agonists may act at the choroid plexus to influence CSF production directly. There are two observations that suggest alpha adrenergic agonists may decrease CSF production. In this study, bilateral cervical sympathetic stimulation reduced the rate of CSF formation 3.0 ± 0.6 µl/min. In addition, when norepinephrine is in the perfusate during ventriculocisternal perfusion, CSF production is decreased approximately 24% (Vates et al.,

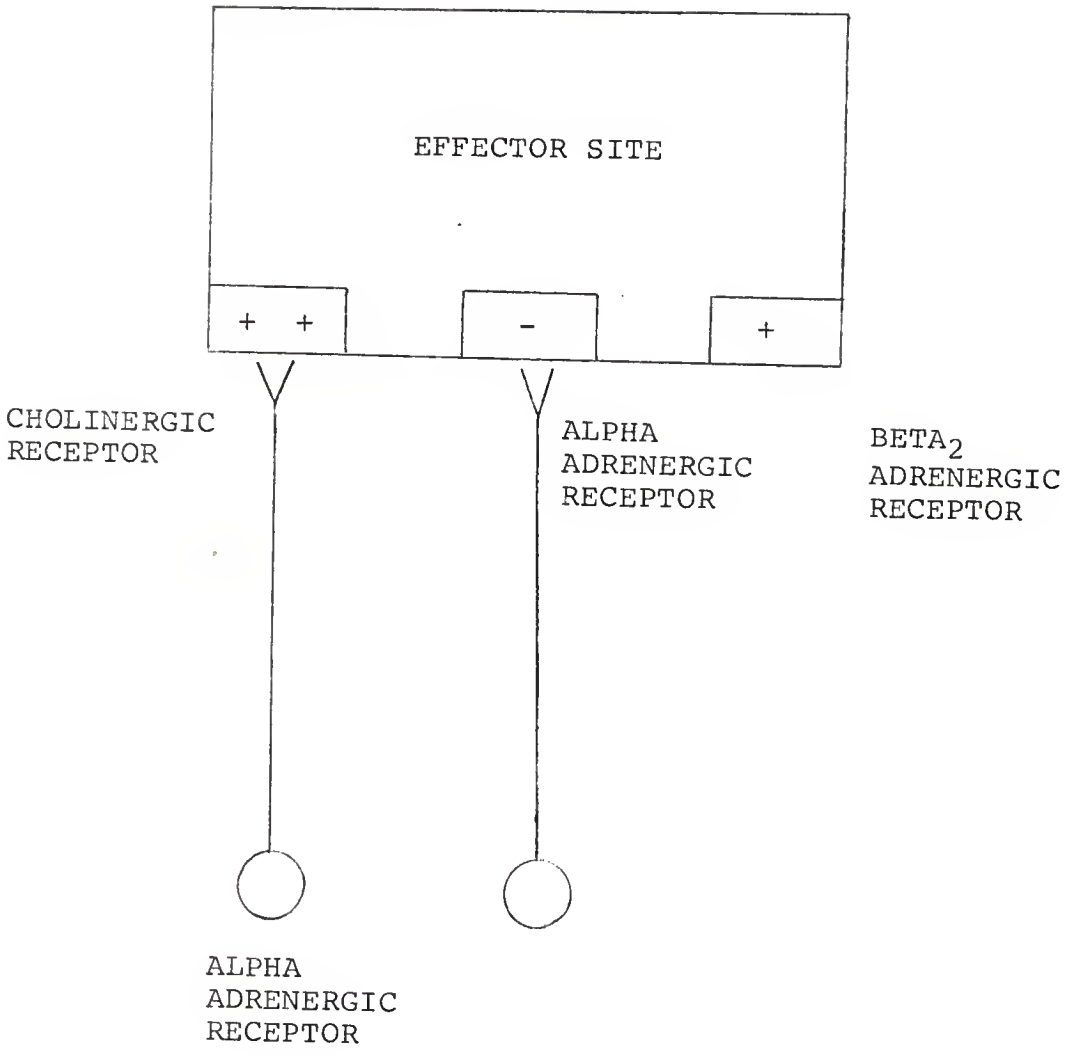
Figure 12. Autonomic pathways to the choroid plexus II. In addition to the cholinergic pathway, the effector site also has a $beta_2$ adrenergic receptor. (+) indicates that activation of these receptors also increases CSF production, but to a lesser extent than cholinergic stimulation.


EFFECTOR SITE
+ +
+
CHOLINERGIC
RECEPTOR
BETA2
ADRENERGIC
RECEPTOR
ALPHA
ADRENERGIC
RECEPTOR

1964). A proposed alpha adrenergic pathway, completing the autonomic innervation of the choroid plexus, is depicted in Figure 13. However, any direct decrease an alpha adrenergic agonist might cause at the choroid plexus would probably be masked by the simultaneous stimulation of production by the cholinergic pathway as described above. Since atropine itself affects the rate of CSF formation, no attempt was made to block the cholinergic receptors and then study the alpha adrenergic system.

Although the sympathetic nervous system has a greater effect on fluid production in other secretory systems than it has on CSF formation, the actions are variable depending on the tissue. Alpha adrenergic agonists increase aqueous humor production by the ciliary body through an increase in arterial blood pressure (Macri et al., 1974). $Beta_2$ agonists, on the other hand, decrease aqueous humor formation (Langham, 1976). In the salivary gland, both alpha and beta adrenergic agonists increase fluid production (Emmelin, 1967). However, the two systems influence secretion in different ways: alpha adrenergic stimulation causes a secretion that is watery and contains high potassium, whereas beta stimulation causes secretion that is viscous and contains digestive enzymes. In the pancreas both norepinephrine and epinephrine decrease secretion; specific beta agonists such as isoproterenol have not been tested (Thomas, 1967).

Figure 13. Autonomic pathways to the choroid plexus III. An alpha adrenergic receptor may also be at the effector site in addition to the cholinergic receptor and $beta_2$ adrenergic receptor. (-) indicates that this pathway decreases the production of CSF.


EFFECTOR SITE
+ +
-
+
CHOLINERGIC RECEPTOR
ALPHA ADRENERGIC RECEPTOR
BETA2 ADRENERGIC RECEPTOR
ALPHA ADRENERGIC RECEPTOR

These secretory tissues derive their alpha adrenergic innervation through the ganglia of the sympathetic chain as does the choroid plexus.

The relatively minor physiological role of the autonomic nervous system in the normal production of CSF is best demonstrated by observing the effects of the autonomic blocking agents. Atropine, the cholinergic blocking agent, decreased the rate of CSF formation 2.6 ± 0.5 µl/min indicating the cholinergic system may continually stimulate fluid secretion but only to the extent of about 15 to 20% of the fluid produced. Phentolamine also reduced CSF formation. This decrease may be occurring through alpha adrenergic blockade of the cholinergic pathway since the change in CSF production is approximately the same as that caused by atropine; however, this is not confirmed. The beta adrenergic system may have little direct function in the normal secretory processes of the choroid plexus since propranolol did not influence CSF production. Nevertheless, it is possible that in unanesthetized animals the autonomic nervous system may exert a greater influence on normal fluid production than is observed here or may cause temporary fluctuations in the rate of fluid formation.

Mechanism of Changes in CSF Production by Autonomic Influences

In two previous investigations, epinephrine and pilocarpine were shown to cause an increase in the flow of CSF from the cisterna magna followed shortly by a reduction in CSF outflow (Becht and Gunnar, 1921; Bhattacharya and Feldberg, 1958). These authors suggested that epinephrine and pilocarpine increased cerebral blood flow and the resulting increase in brain volume forced subarachnoid CSF out through the cisternal cannula without affecting true CSF production. Increased volumes were also observed in response to the autonomic agonists used in the study presented in this dissertation. The increased rate of outflow during drug treatment averaged approximately 35 µl/min over control for a three minute collection period. If pressure on the subarachnoid fluid was a partial cause of this phenomenon, the additional fluid collected would have come first from the cisterna magna and basal cisterns which contained the dye marker at the same dilution as the control perfusate. Hence, any error in the calculation of production changes as a result of this would cause an underestimate of the dilution of the inflowing perfusate and an underestimate of the actual increase in fluid production. By measuring only volumes, the early investigators could not reliably show whether the agents actually increased fluid formation. The

increases in CSF production calculated from dye dilution in this study indicate that autonomic agents do alter fluid formation.

The increase in CSF production caused by cholinergic and adrenergic agonists can be ascribed to two possible mechanisms of action, depending on the effector site. Either a change in the hemodynamic relationships or a direct action on the secretory cells might result in alterations in CSF formation by these drugs. An increase in the supply of blood to the brain or choroid plexus would provide more fluid to produce an ultrafiltrate of plasma in the CSF. Such a transfer of plasma water would increase CSF volume.

Peripheral blood pressure changes do not correlate well with CSF production changes. Carbachol and salbutamol decrease blood pressure; this could decrease the supply of blood to the secretory tissue. Phenylephrine, on the other hand, increases peripheral blood pressure which might serve to increase blood flow to the brain and choroid plexus and thus to increase the rate of CSF formation. However, this alpha adrenergic agonist also increases blood pressure during treatment with atropine and hemicholinium which block the increased CSF production by phenylephrine.

Since blood pressure changes were measured peripherally, choroid plexus and cerebral blood flow data were considered necessary to determine possible vascular actions

of these agents at the site of CSF formation. The control blood flow to the choroid plexus measured by microspheres was 2.26 ± 0.28 ml/gm min. This flow rate is comparable to 3.01 ml/gm min observed by Alm and Bill (1973) for the choroid plexus using the same technique. It is also similar to the estimates of choroid plexus blood flow by Welch (1963) and Pollay et al. (1972). The flow to the choroid plexus represents one of the greatest amounts of blood per tissue weight delivered to any tissue. Carbachol and phenylephrine increased mean plexus blood flow 0.81 and 0.77 ml/gm min, respectively. Salbutamol increased blood flow to the choroid plexus 0.35 ml/gm min. The effects of these drugs on blood flow are in about the same proportions as their effects on CSF production. However, none of these changes in choroid plexus blood flow differ statistically from control flows because of the wide variation in the changes. These data, along with the data of Alm and Bill (1973) showing no effect on plexus blood flow by sympathetic stimulation, seem to indicate that the autonomic nervous system does not significantly alter the blood flow to the choroid plexus.

Cerebral blood flow in the control experiments was 0.46 ± .04 ml/gm min. This value is approximately the same as the flows measured for the dog (Marcus et al., 1976) and sheep (Hales, 1973). There was no alteration in brain blood flow after carbachol, phenylephrine, or

salbutamol. When Salanga and Waltz (1973) stimulated the facial nerve, vasodilation occurred only if the nerve was cut where it left the brainstem. This suggests that sensory nerve fibers detecting cerebral vasodilation might pass back into the central nervous system through the facial nerve causing a reflex vasoconstriction. If such a pathway does exist, it would also explain the ineffectiveness of salbutamol and carbachol to increase cerebral blood flow. The inability of phenylephrine to alter cerebral blood flow is probably due to its low efficacy on alpha adrenergic receptors in cerebral blood vessels (Duckles and Bevan, 1976). Blood pressure and blood flow data seem to indicate that the autonomic treatments performed in this study produce minimal effects on the production of CSF by way of changes in blood flow. Also, it is clear that any increase in the rate of outflow seen during drug treatment was not mediated by a generalized increase in blood flow to the brain. An increase in choroid plexus blood flow could have forced additional perfusate from the ventricles.

Verapamil and theophylline were used to investigate two possible ways in which autonomic agents could influence CSF production by direct action on secretory cells. The presence of calcium is a requirement for cholinergic stimulation of salivary secretion (Douglas and Poisner, 1963). If calcium influx has a role in increasing CSF

production stimulated by carbachol, then verapamil, which blocks calcium channels (Kohlhardt et al., 1972), should prevent the increase in fluid formation caused by the cholinergic agonist. Verapamil has been shown to be effective when administered on either side of the epithelium and was given intraventricularly (Bentley, 1974). Verapamil did not affect either normal CSF formation or fluid production stimulated by carbachol; therefore, fluid secretion probably occurs through a mechanism other than a calcium-induced activation.

Since the effects of both cholinergic and adrenergic neurotransmitters may be mediated by changes in cyclic nucleotide levels (Greengard, 1976), an increase in guanosine 3',5' cyclic monophosphate (cGMP) or adenosine 3',5' cyclic monophosphate (cAMP) might be the means by which carbachol, phenylephrine, and salbutamol increase the production of CSF. Theophylline, which inhibits the destruction of the nucleotides by phosphodiesterase among other actions, was used to enhance the levels of the cyclic nucleotides. These experiments showed that theophylline caused a significant dose-dependent increase in CSF formation. The higher dose increased fluid production 9.2 ± 1.4 µl/min which is approximately equal to the maximum effect of the autonomic agonists. Unfortunately, theophylline is not totally specific for either cGMP or cAMP phosphodiesterases (Amer and Kreighbaum,

1975), so it is not possible to show whether the effects mediated by cyclic nucleotide action are cholinergic, $beta_2$ adrenergic, or both. Whereas the theophylline experiments suggest that increases in cyclic nucleotide levels are responsible for the increases in fluid production elicited by carbachol, phenylephrine, and salbuatmol administration, they do not provide conclusive evidence that enhancement of the cyclic nucleotide concentrations takes place in choroid plexus epithelia. However, the relatively large blood flow to the choroid plexus does not significantly change during treatment with any of the autonomic agents that increase CSF production. Apparently, the blood flow only provides fluid and electrolyte substrate for the secretory processes of the choroid plexus epithelium. The data strongly imply that autonomic agents influence CSF production by causing changes primarily at the choroid plexus epithelium.

The changes in CSF production mediated by the epithelial cells are probably a result of alterations in the transport of electrolytes. Three pathways have been demonstrated for the secretory transfer of electrolytes in the CSF: the catalysis of bicarbonate formation by carbonic anhydrase, the transport of cations by the ouabain-sensitive sodium-potassium ATPase, and the probable transport of chloride by an ethacrynic acid-sensitive pump. Inhibition of any of the three mechanisms

has been shown to decrease the rate of CSF formation. Inhibition of carbonic anhydrase by acetazolamide, benzolamide, or methazolamide reduces fluid production approximately 50 to 70% (Pollay and Davson, 1963; Davson and Segal, 1970; Broder and Oppelt, 1969; and this study). That this effect involves the movement of bicarbonate ions into CSF was first demonstrated by Maren and Broder (1970) and further quantified by Vogh and Maren (1975). Ouabain decreases the production of CSF 50 to 60% by its inhibition of sodium-potassium ATPase (Vates et al., 1964; Davson and Segal, 1970; Garg and Mathur, 1975). A 50 to 60% reduction in CSF formation is seen with ethacrynic acid (Domer, 1969; Miner and Reed, 1971) and furosemide (McCarthy and Reed, 1974). Active transfer of chloride ions inhibited by ethacrynic acid and furosemide has been described for the ascending limb of Henle's loop in the kidney (Burg, 1974), and thus may be presumed to occur in CSF secretion. The possibility of an active chloride pump in the choroid plexus is supported by the findings that the chloride concentration in the CSF can be maintained during reduced plasma levels of the ion (Abbott et al., 1971; Bourke et al., 1970).

Assuming that changes in cGMP are associated with cholinergic responses as in other tissues (Greengard, 1976), levels of cGMP may be increased at the critical site for CSF production by cholinergic agonists or by alpha adrenergic stimulation of the cholinergic pathway

and decreased by inhibition of the cholinergic pathway. Concentrations of cAMP, on the other hand, generally change in response to agents that affect the adrenergic receptors. Therefore cAMP may be increased by $beta_2$ stimulation and decreased by direct activation of the alpha adrenergic receptors. The cholinergic pathway appears to predominate over the direct adrenergic pathway since activation of the cholinergic receptors causes a greater increase in CSF production, and inhibition of cholinergic receptors, but not $beta_2$ adrenergic receptors, causes a decrease in CSF formation. Any increase in concentration of either cyclic nucleotide is presumed to activate a protein kinase system which stimulates the secretion of electrolytes and water through one of the possible transport pathways.

The transport mechanism that the autonomic nervous system affects through the cyclic nucleotides is still unknown. The cyclic nucleotides may be activating one or more of the transport systems described or systems not yet discerned. However, some data on this matter were collected in this study for the carbonic anhydrase system. During carbonic anhydrase inhibition, carbachol and phenylephrine increased CSF production approximately the same amount as when the drugs were given alone. This would support the possibility that autonomic agents acted through a separate mechanism. On the other hand, although

atropine and phentolamine decreased CSF formation when given alone, neither caused a further reduction in combination with methazolamide. This would seem to indicate that the pathway for carbonic anhydrase inhibitors and inhibitors of the cholinergic pathway are the same or overlapping. However, it should be noted that the dose of methazolamide given decreased the rate of CSF production to about 8 µl/min. This is probably the maximal reduction possible since none of the agents that have been tested can decrease the rate of formation below 6 to 9 µl/min in cats (Vates et al., 1964; Garg and Mathur, 1975). This baseline production may be accounted for by diffusion across the ventricular ependyma (Milhorat, 1972). If submaximal doses of a carbonic anhydrase inhibitor are combined with an inhibitor of another transport system, additive effects in reducing CSF formation might be seen down to 6 to 9 µl/min (Garg and Mathur, 1975).

Regardless of which transport system(s) the autonomic nervous system control(s) through cyclic nucleotide levels, the role of the nucleotides is probably to serve as activators for the enhancement of fluid secretion above normal. The reduction in CSF production by atropine, phentolamine, or cervical sympathetic stimulation was only 15 to 20% of control, indicating a relatively minor role in the normal function of the transport system. This is especially small when compared to the ability of specific transport inhibitors to decrease

the rate of CSF formation by 50 to 70%. On the other hand, the autonomic agonists and theophylline were shown to increase CSF production from 15 to 20 µl/min to 25 to 30 µl/min, an increment of 50 to 70% of control.

Practical Significance

These experiments have suggested that the changes mediated by the autonomic agonists probably result from a direct activation of the choroid plexus epithelium. They have also disclosed a unique system in which alpha adrenergic agents stimulate a cholinergic pathway to increase fluid formation. This study does not resolve the question of how CSF is secreted or even exactly how the autonomic agents influence fluid formation; however, the data do contribute to the understanding of a very complex secretory system.

The physiological significance of autonomic function in the production of CSF remains uncertain. The basal rate of CSF formation may be partially dependent upon autonomic nervous activity. Receptors for autonomic agonists may play an important role in bringing about fluctuation in CSF production; however, the value of such fluctuations is not clear at this time.

One of the original goals of this study was to seek an agent that acted through the autonomic nervous sytem to decrease the production of CSF. Such an agent might

then be used alone or in addition to carbonic anhydrase inhibitors in the therapy of hydrocephalus caused by an increased secretion of CSF or as an adjunct to the treatment of obstructive hydrocephalus. Failure to achieve additive effects from methazolamide and blockade of the cholinergic pathway does not necessarily mean that autonomic agents have no role in the treatment of hydrocephalus. In this pathologic situation, reduction of cholinergic stimulation to production may be highly beneficial if this stimulation is excessive. Furthermore, if plasma catecholamines are elevated during increased intraventricular volume as may be suggested by the observation that hypertensive cerebrovascular disease sometimes accompanies normal pressure hydrocephalus (Earnest et al., 1974), an alpha adrenergic receptor blocking agent or perhaps atropine may be useful in reducing new CSF formation.

Although the physiological and clinical significance of the data presented in this dissertation is uncertain, this study does provide important basic information for the design of future experiments on anatomical organization of nerve pathways, on secretory mechanisms, and on practical application in human disease.

APPENDIX I

Autonomic Innervation and Function of Cerebral Blood Vessels

The innervation of cerebral blood vessels has been identified according to sympathetic and parasympathetic function. Forbes and Wolf (1928) demonstrated a sympathetic influence on cerebral blood vessels by stimulating the cervical sympathetic trunk and observing a constriction of pial vessels. More recently, investigators have used fluorescence microscopy to confirm the presence of adrenergic fibers to several pial vessels including the circle of Willis, the anterior cerebral artery, and the middle of cerebral artery (Falck et al., 1968; Nielsen and Owman, 1967). Fluorescence disappears from these vessels after a cervical sympathetic ganglionectomy. Adrenergic endings on pial blood vessels have also been shown with electron microscopy in which the sympathetic terminals can be identified as containing small granular vesicles (Iwayama et al., 1970; Nielsen et al., 1971). The small parenchymal vessels within the brain, however, have an alternative source of adrenergic innervation which originates in the brainstem rather than the cervical sympathetic chain. After a bilateral sympathectomy,

Hartman (1973) detected the continuing prescence of dopamine-β-hydroxylase, the enzyme responsible for norepinephrine synthesis, in the deep vessels of the brain by immunofluorescence, and Edvinsson et al. (1973a) found persistent fluorescence (indicating norepinephrine) in the vessels supplying the inner structures. Raichle et al. (1975) have suggested that these nerves arise from the locus coelruleus since carbachol applied to the structure bilaterally causes a significant decrease in cerebral blood flow.

The parasympathetic innervation of the cerebral vasculature is also well established. Forbes and Wolf (1928) stimulated the vagus and observed cerebral vasodilatation. However, this was shown to be due to a secondary activation of the facial cranial nerve in the medulla (Chorobski and Penfield, 1932). The presence of cholinergic fibers in pial vessels was confirmed by acetylcholinesterase staining (Edvinsson et al., 1972a). Electron microscopists have observed nerve terminals in pial vessels containing small agranular vesicles and a few large granular vesicles which are characteristic of cholinergic fibers (Edvinsson et al., 1972a; Iwayama et al., 1970; Motavkin and Osipova, 1973; Nielsen et al., 1971). Edvinsson (1975) has also performed experiments in which the intracranial parts of the cranial nerves were cut, and found no loss of acetylcholinesterase staining in

blood vessels occurring within two weeks. This seems to indicate a source of cholinergic innervation other than the facial nerve.

Some investigators have been able to show changes in cerebral blood flow (CBF) by electrical stimulation of the nerves supplying the blood vessels or by agents that affect autonomic receptors. Most of the work investigating sympathetic control of cerebral blood vessels has involved stimulation of the cervical sympathetic trunk. Forbes and Wolf (1928) first showed that sympathetic stimulation constricted pial blood vessels. Later it was demonstrated that the constriction could be abolished with cocaine, an agent that prevents norepinephrine reuptake, or ergotamine, an alpha adrenergic blocking agent (Forbes and Cobb, 1938). More recently, an electromagnetic flowmeter at the internal maxillary artery was used to measure a decrease in CBF during sympathetic stimulation; this was blocked by phentolamine (Lluch et al., 1975). D'Alecy and Feigl (1972) also demonstrated a reduction in flow using a flowmeter. On the other hand, Raper et al. (1972) could not show any vasoconstriction with sympathetic stimulation, and no change in CBF was observed with the microsphere technique during cervical sympathetic stimulation (Alm and Bill, 1973). Two studies have been found in which norepinephrine was given. Meyer and Welch (1972) observed a constriction of the cerebral vessels whereas Raper et al. (1972) did not detect any change in vessel diameter.

Investigation of parasympathetic control of cerebral blood vessels has been less extensive, but the investigators seem to be in agreement that vasodilation does result from parasympathetic stimulation. After Chorobski and Penfield (1932) demonstrated dilation of cerebral vessels by stimulating the facial nerve at the geniculate ganglion, it was discovered that atropine could block the action (Forbes and Cobb, 1938). More recently, Salanga and Waltz (1973) confirmed the increase in CBF with stimulation of the facial nerve after the nerve had been severed from the brainstem. Acetylcholine, administered intravenously or intra-arterially, also increased cerebral blood vessel diameter (Carpi, 1972).

In vitro studies have been useful in characterizing autonomic cerebrovascular responses. Bevan and Bevan (1973) found that the maximal contraction of the rabbit basilar artery with electrical stimulation *in vitro* was about 25% of the maximum for the ear artery. Alpha adrenergic agonists have also been studied in isolated vessels. Several investigators have demonstrated that norepinephrine contracts cerebral vessels *in vitro*; however, the response of large cerebral vessels to norepinephrine is less than can be observed with other agents such as serotonin (Nielsen and Owman, 1971; Toda and Fujita, 1973; Dalske et al., 1974; Duckles and Bevan, 1976). Duckles and Bevan (1976) also noted that the

rabbit basilar artery showed a biphasic dose-response constriction following alpha adrenergic agonists with both phases having sigmoidal log dose-response curves. One curve ranged from 10^{-6} to 10^{-4} M and reached a plateau, then the other curve started at about 10^{-4} M and reached a maximum at 10^{-2} M. In both sensitive portions of the dose-response curve, phenylephrine showed only one-tenth the potency of l-norepinephrine. The authors suggest that the response to the lower doses represents the physiological response while the response to higher doses represents the activation of a non-adrenergic receptor that can be non-specifically elicited by high concentrations of an adrenergic agonist. This interpretation may also draw support from the observations of Nielsen and Owman (1971) and Duckles and Bevan (1976) that high concentrations of isoproterenol can contract the vascular smooth muscle. The existence of beta adrenergic receptors in the cerebral vasculature seems to vary with species. So far the rabbit and the cat have been tested with isoproterenol, and only the cat showed a vasodilator response. When cat cerebral vessels were contracted *in vitro* with serotonin, both isoproterenol and tertbutaline relaxed the vessels, and the effect was blocked by propranolol (Edvinsson and Owman, 1974). Acetylcholine (10^{-8} to 10^{-6} M) also relaxed cerebral vessels contracted *in vitro* (Edvinsson, 1975).

APPENDIX II

Composition of Artificial Cerebrospinal Fluid [modified from Vates et al., (1964)]

Salt	mM
NaCl	130.0
KCl	2.5
$MgSO_4$	1.0
NaH_2PO_4	0.5
Na_2HPO_4	0.5
$CaCl_2$	2.5
$NaHCO_3$	20.0

2.5 mg/ml of blue dextran was added immediately prior to each experiment

REFERENCES

Abbott, J., Davson, H., Glen, I., and Grant, N. Chloride transport and potential across the blood-cerebrospinal fluid barrier. *Brain Res.*, 29: 185-193, 1971.

Alm, A. and Bill, A. The effect of stimulation of the cervical sympathetic chain on retinal oxygen tension and on uveal, retinal and cerebral blood flows in cats. *Acta Physiol. Scand.*, 88: 84-94, 1973.

Amer, M.S. and Kreighbaum, W.E. Cyclic nucleotide phosphodiesterases: Properties, activators, inhibitors, structure-activity relationships, and possible role in drug development. *J. Pharmacol. Sci.*, 64: 1-37, 1975.

Becht, F.C. and Gunnar, H. Studies on the CSF. VII. A study of volume changes of the CSF after adrenalin, pituitrin, pilocarpin, and atropin. *Amer. J. Physiol.*, 56: 231-240, 1921.

Benedikt, M. Uber die Innervation des Plexus chorioideus inferior. *Virchows Arch. Path. Anat.*, 59: 395-400, 1874.

Bentley, P.J. Effects of verapamil on the short-circuit current of an epithelial membrane: The toad urinary bladder. *J. Pharmacol. Exp. Ther.*, 189: 563-569, 1974.

Bevan, J.A. and Bevan, R.D. Localized neurogenic vasoconstriction of the basilar artery. *Stroke*, 4: 760-763, 1973.

Bhattacharya, B.K. and Feldberg, W. Perfusion of cerebral ventricles. Effects of drugs on outflow from the cisterna and the aqueduct. *Brit. J. Pharmacol.*, 13: 156-162, 1958.

Bourke, R.S., Gabelnick, H.L., and Young, O. Mediated transport of chloride from blood into cerebrospinal fluid. *Exp. Brain Res.*, 10: 17-38, 1970.

Broder, L.E. and Oppelt, W.W. Effect of benzolamide on cerebrospinal fluid formation. *J. Pharmacol. Exp. Ther.*, 169: 271-276, 1969.

Buckberg, G.D., Luck, J.C., Payne, D.B., Hoffman, J.I.E., Archie, J.P., and Fixler, D.E. Some sources of error in measuring regional blood flow with radioactive microspheres. *J. Appl. Physiol.*, 31: 598-604, 1971.

Burg, M.B. The mechanism of action of diuretics in renal tubules. In: *Recent Advances in Renal Physiology and Pharmacology*, ed. by L.G. Wesson and G.M. Fanelli, pp. 99-109, Univ. Park Press, Baltimore, 1974.

Carpi, A. Drugs related to chemical mediators. In: *Pharmacology of the Cerebral Circulation, International Encyclopedia of Pharmacology and Therapeutics*, vol. I, 87-124, Pergamon Press, New York, 1972.

Chiou, C.Y. and Zimmerman, T. Ocular hypotensive effects of autonomic drugs. *Invest. Ophthal.*, 14: 416-417, 1975.

Chorobski, J. and Penfield, W. Cerebral vasodilator nerves and their pathway from the medulla oblongata with observations on the pial and intracerebral vascular plexuses. *Arch. Neurol. and Psychiat.*, 28: 1257-1289, 1932.

Clark, S.L. Nerve endings in the choroid plexus of the fourth ventricle. *J. Comp. Neurol.*, 47: 1-16, 1928.

Clark, S.L. Innervation of the choroid plexuses and the blood vessels within the central nervous system. *J. Comp. Neurol.*, 60: 21-31, 1934.

Cullum, V.A., Farmer, J.B., Jack, D., and Levy, G.P. Salbutamol: A new, selective beta-adrenoceptive receptor stimulant. *Brit. J. Pharmacol.*, 35: 141-151, 1969.

D'Alecy, L.G. and Feigl, E.O. Sympathetic control of cerebral blood flow in dogs. *Circ. Res.*, 31: 267-283, 1972.

Dalske, H.F., Harakal, C., Sevy, R.W., and Menkowitz, B.J. Catecholamine content and response to norepinephrine of middle cerebral artery. *Proc. Soc. Exp. Biol. Med.*, 146: 718-721, 1974.

Davson, H. *Physiology of the Cerebrospinal Fluid*, Little, Brown and Co., Boston, 1967.

Davson, H. and Segal, M.B. The effects of some inhibitors and accelerators of sodium transport on the turnover of ^{22}Na in the cerebrospinal fluid and brain. *J. Physiol. (Lon.)*, 209: 131-153, 1970.

Domer, F.R. Effects of diuretics on CSF formation and potassium movement. *Exp. Neurol.*, 24: 54-64, 1969.

Douglas, W.W. and Poisner, A.M. The influence of calcium on the secretory response of the submaxillary gland to acetylcholine or to adrenaline. *J. Physiol. (Lon.)*, 165: 528-541, 1963.

Duckles, S.P. and Bevan, J.A. Pharmacological characterization of adrenergic receptors of a rabbit cerebral artery *in vitro*. *J. Pharmacol. Exp. Ther.*, 197: 371-378, 1976.

Earnest, M.P., Fahn, S., Karp, J.H., Rowland, L.P. Normal pressure hydrocephalus and hypertensive cerebrovascular disease. *Arch. Neurol.*, 31: 262-266, 1974.

Edvinsson, L. Neurogenic mechanisms in the cerebrovascular bed. Autonomic nerves, amine receptors and their effects on cerebral blood flow. *Acta Physiol. Scand.*, Suppl. 427: 5-35, 1975.

Edvinsson, L., Lindvall, M., Nielsen, K.C., and Owman, C. Are brain vessels innervated also by central (non-sympathetic) adrenergic neurons? *Brain Res.*, 63: 496-499, 1973a.

Edvinsson, L., Nielsen, K.C., and Owman, C. Cholinergic innervation of the choroid plexus in rabbits and cats. *Brain Res.*, 63: 500-503, 1973b.

Edvinsson, L., Nielsen, K.C., Owman, C., and Sporrang, B. Cholinergic mechanisms in pial vessels. Histochemistry, electron microscopy, and pharmacology. *Z. Zellforsch.*, 134: 311-325, 1972a.

Edvinsson, L., Nielsen, K.C., Owman, C., and West, K.A. Adrenergic innervation of the mammalian choroid plexus. *Amer. J. Anat.*, 139: 299-308, 1974.

Edvinsson, L. and Owman, C. Pharmacological characterization of adrenergic alpha and beta receptors mediating the vasomotor responses of cerebral arteries *in vitro*. *Circ. Res.*, 35: 835-849, 1974.

Edvinsson, L., Owman, C. Rosengren, E., and West, K.A. Concentration of norepinephrine in pial vessels, choroid plexus, and iris during two weeks after sympathetic ganglionectomy or decentralization. *Acta Physiol. Scand.*, 85: 201-206, 1972b.

Ellis, P.P. Pilocarpine therapy. *Survey Ophthal.*, 16: 165-169, 1971.

Emmelin, N. Nervous control of salivary glands. In: *Handbook of Physiology*, Section 6: Alimentary Canal, vol. II: Secretion, ed. by C.F. Code, pp. 595-632, Amer. Physiol. Soc., Washington, D.C., 1967.

Falck, B., Nielsen, K.C., Owman, C. Adrenergic innervation of the pial circulation. *Scand. J. Clin. Lab. Invest.*, Suppl. 102: 96-98, 1968.

Findlay, J.W. The choroid plexuses of the lateral ventricles of the brain, their histology, normal and pathological, (in relation specially to insanity). *Brain*, 22: 161-202, 1899.

Forbes, H.S. and Cobb, S.S. Vasomotor control of cerebral vessels. *Brain*, 61: 221-233, 1938.

Forbes, H.S. and Wolf, H.G. Cerebral circulation. III. The vasomotor control of cerebral vessels. *Arch. Neurol. and Psychiat.*, 19: 1056-1086, 1928.

Foster, R.W. Adrenoreceptors and salt and water movement in epithelia. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, 281: 315-326, 1974.

Gardiner, J.E. Experiments on the site of action of a choline acetylase inhibitor. *J. Physiol. (Lon.)*, 138: 13P-14P, 1957.

Garg, L.C. and Mathur, P.P. Effect of ouabain on cerebrospinal fluid formation after carbonic anhydrase inhibition. *Arch. Int. Pharmacodyn. Ther.*, 213: 190-194, 1975.

Goldstein, A. *Biostatistics*, Macmillan Co., London, 1964.

Greengard, P. Possible role for cyclic nucleotides and phosphorylated membrane proteins in post-synaptic actions of neurotransmitters. *Nature*, 260: 101-108, 1976.

Hales, J.R.S. Radioactive microsphere measurement of cardiac output and regional tissue blood flow in the sheep. *Pflugers Arch. ges. Physiol.*, 344: 119-132, 1973.

Hartman, B. The innervation of cerebral blood vessels by central noradrenergic neurons. In: *Frontiers in Catecholamine Research*, eds. E. Usdin and S. Snyder, pp. 91-96, Pergamon Press, New York, 1973.

Heisey, S.R., Held, D., and Pappenheimer, J.R. Bulk flow and diffusion in the cerebrospinal fluid system of the goat. *Amer. J. Physiol.*, 203: 775-781, 1962.

Iwayama, T., Furness, J.B., and Burnstock, G. Dual adrenergic and cholinergic innervation of the cerebral arteries of the cat. *Circ. Res.*, 26: 635-645, 1970.

Jard, S. Adrenergic receptors in epithelia. In: *Drugs and Transport Processes*, ed. by B.A. Callingham, pp. 111-128, Univ. Park Press, Baltimore, 1974.

Junet, W. Terminaisons nervenses intra-epitheliales dans les plexus choroides de la souris. *C. R. Soc. Biol. (Paris)*, 95: 1397-1398, 1926.

Koelle, G. Neurohumoral transmission and the autonomic nervous system. In: *The Pharmacological Basis of Therapeutics*, eds. L.S. Goodman and A. Gilman, pp. 402-441, Macmillan Co., New York, 1970.

Kohlhardt, M., Bauer, B., Krause, H., and Fleckenstein, A. New selective inhibitors of the transmembrane calcium conductivity in mammalian myocardial fibers. Studies with the voltage clamp technique. *Experientia (Basel)*, 28: 288-289, 1972.

Langham, M.E. Personal communication. 1976.

Langham, M.E. and Diggs, E. Beta adrenergic responses in the eyes of rabbits, primates, and man. *Exp. Eye Res.*, 19: 281-295, 1974.

Lockwood, R.H. and Lum, B.K.B. Selective adrenergic stimulant actions of 1-isopropylamino-3-(2 thiazoloxy)-2 propanol (ITP) in anesthetized cats. *Life Sci.*, 14: 73-81, 1974.

Lluch, S., Gomoz, B., Alborch, E., Urquilla, P.R. Adrenergic mechanisms in cerebral circulation of the goat. *Amer. J. Physiol.*, 228: 985-989, 1975.

Macri, F.J. Vasoconstriction produced in the iris-ciliary body of the cat eye by stimulation of local ganglion-like receptors. *Invest. Ophthal.*, 10: 581-588, 1971.

Macri, F.J. and Cevario, S.J. The induction of aqueous humor formation by the use of ACh and eserine. *Invest. Ophthal.*, 12: 910-916, 1973.

Macri, F.J., Cevario, S.J., and Ballintine, E.J. The arterial pressure dependency of the increased aqueous humor formation induced by ACh + eserine. *Invest. Ophthal.*, 13: 153-155, 1974.

Macri, F.J., Politoff, A., Rubin, R., Dixon, R., Rall, R. Preferential vasoconstrictor properties of acetazolamide on the arteries of the choroid plexus. *Int. J. Neuropharmacol.*, 5: 109-115, 1966.

Mangos, J.A., McSherry, N.R., and Arvanitakis, S.N. Autonomic regulation of secretion and transductal fluxes of ions in the rat parotid. *Amer. J. Physiol.*, 225: 683-688, 1973.

Marcus, M.L., Heistad, D.D., Ehrhardt, J.C., and Abboud, F.M. Total and regional cerebral blood flow measurement with 7-10, 15, 25, and 50 μm microspheres. *J. Appl. Physiol.*, 40: 501-507, 1976.

Maren, T.H. and Broder, L.E. The role of carbonic anhydrase in anion secretion into cerebrospinal fluid. *J. Pharmacol. Exp. Ther.*, 172: 197-202, 1970.

Martins, A.N., Ramirez, A., Solomon, L.S., and Wiese, G.M. The effect of dexamethasone on the rate of formation of cerebrospinal fluid in the monkey. *J. Neurosurg.*, 41: 550-554, 1974.

Maxwell, D.S. and Pease, D.C. The electron microscopy of the choroid plexus. *J. Biophys. Biochem. Cytol.*, 2: 467-474, 1956.

McCarthy, K.D. and Reed, D.J. The effect of acetazolamide and furosemide on CSF production and choroid plexus carbonic anhydrase activity. *J. Pharmacol. Exp. Ther.*, 189: 194-201, 1974.

Meyer, J.S. and Welch, K.M.A. Relationship of cerebral blood flow and metabolism to neurological symptoms. In: *Progress in Brain Research: Cerebral Blood Flow*, ed. by J.S. Meyer, pp. 285-347, Elsevier Publ. Co., New York, 1972.

Milhorat, T.H. *Hydrocephalus and Cerebrospinal Fluid*, Williams and Wilkins Co., Baltimore, 1972.

Millen, J.W. and Rogers, G.E. An electron microscopic study of the choroid plexus in the rabbit. *J. Biophys. Biochem. Cytol.*, 2: 407-416, 1956.

Miner, L.C. and Reed, D.J. The effect of ethacrynic acid on sodium uptake into the cerebrospinal fluid of the rat. *Arch. Int. Pharmacodyn. Ther.*, 190: 316-321, 1971.

Mitchell, G.A.G. The parasympathetic component. In: *Anatomy of the Autonomic Nervous System*, E. and S. Livingstone, Ltd., London, 1953.

Motvakin, P.A. and Osipova, L.P. Cholinergic innervation of the human brain arterioles. *Z. Mikrosk. Anat. Forsch.*, 87: 365-378, 1973.

Nielsen, K.C. and Owman, C. Adrenergic innervation of pial arteries related to the circle of Willis in the cat. *Brain Res.*, 6: 773-776, 1967.

Nielsen, K.C. and Owman, C. Contractile response and amine receptor mechanisms in isolated middle cerebral artery of the cat. *Brain Res.*, 27: 33-42, 1971.

Nielsen, K.C., Owman, C., and Sporrang, B. Ultrastructure of the autonomic innervation apparatus in the main pial arteries of rats and cats. *Brain Res.*, 27: 25-32, 1971.

Oppelt, W.W., Patlak, C.S., and Rall, D.P. The effect of certain drugs on cerebrospinal fluid production in the dog. *Amer. J. Physiol.*, 206: 247-250, 1964.

Paterson, G.D. and Paterson, G. Drug therapy of glaucoma. *Brit. J. Ophthal.*, 56: 288-294, 1972.

Petersen, O.H. Acetylcholine-induced ion transports involved in the formation of saliva. *Acta Physiol. Scand.*, Suppl. 381: 9-57, 1972.

Politoff, A. and Macri, F.J. Pharmacological differences between isolated, perfused arteries of the choroid plexus and of the brain parenchyma. *Int. J. Neuropharmacol.*, 5: 155-162, 1966.

Pollay, M.A. and Davson, H. The passage of certain substances out of the cerebrospinal fluid. *Brain*, 86: 137-150, 1963.

Pollay, M.A., Stevens, A., Estrada, E., and Kaplan, R. Extracorporeal perfusion of choroid plexus. *J. Appl. Physiol.*, 32: 612-617, 1972.

Raichle, M.E., Hartman, B.K., Eichling, J.O., and Sharpe, L.G. Central noradrenergic regulation of cerebral blood flow and vascular permeability. *Proc. Nat. Acad. Sci.*, 72: 3726-3730, 1975.

Raper, A.J., Kontos, H.A., Wei, E.P., and Patterson, J.L. Unresponsiveness of pial precapillary vessels to catecholamines and sympathetic nerve stimulation. *Circ. Res.*, 31: 257-266, 1972.

Salanga, V.D. and Waltz, A.G. Regional cerebral blood flow during stimulation of seventh cranial nerve. *Stroke*, 4: 213-217, 1973.

Snedecor, G.W. and Cochran, W.G. *Statistical Methods*, 6th ed., Iowa State Univ. Press, Ames, 1967.

Stohr, P.J. Uber die Innervation der Pia mater und des Plexus chorioideus des Menschen. *Zietschr. f. Anat. und Entwickl.*, 63: 562-607, 1922.

Tennyson, V. Ultrastructural characteristics of the telencephalic and myelencephalic choroid plexuses in fetus of man and rabbit, and a comparison with the adult choroid plexus in rabbit. In: *The Choroid Plexus in Health and Disease*, eds. M.G. Netsky and S. Shuangshoti, pp. 36-72, Univ. of Virginia Press, Charlottesville, 1975.

Thomas, J.E. Neural regulation of pancreatic secretion. In: *Handbook of Physiology*, Section 6: Alimentary Canal, vol. II: Secretion, ed. by C.F. Code, pp. 955-968, Amer. Physiol. Soc., Washington, D.C., 1967.

Toda, N. and Fujita, Y. Responsiveness of isolated cerebral and peripheral arteries to serotonin, norepinephrine, and transmural electrical stimulation. *Circ. Res.*, 33: 98-104, 1973.

Tsuker, M. Innervation of the choroid plexus. *Arch. Neurol. Psychiat.*, 58: 474-483, 1947.

Vates, T.S., Bonting, S.L., and Oppelt, W.W. Sodium-potassium activated adenosine triphosphate formation of cerebrospinal fluid in the cat. *Amer. J. Physiol.*, 206: 1165-1172, 1964.

Vogh, B.P. and Maren, T.H. Sodium, chloride, and bicarbonate movement from plasma to cerebrospinal fluid in cats. *Amer. J. Physiol.*, 228: 673-683, 1975.

Wagner, H.N., Rhodes, B.A., Sasaki, Y. and Ryan, J.P. Studies of the circulation with radioactive microspheres. *Invest. Radiol.*, 4: 374-386, 1969.

Welch, K. Secretion of cerebrospinal fluid by choroid plexus of rabbits. *Amer. J. Physiol.*, 205: 617-624, 1963.

BIOGRAPHICAL SKETCH

Joseph Roscoe Haywood was born on May 29, 1949 in Columbus, Ohio. He attended the Wilmington, Ohio City School System and graduated from Wilmington Senior High School in 1967. He continued his education at the University of Kentucky in Lexington, Kentucky where he received a Bachelor of Science degree in zoology in 1971. That same year he started graduate work in the Thomas Hunt Morgan School of Biological Sciences at the University of Kentucky and obtained a Master of Science degree in zoology in 1972. The author continued his graduate study leading to the degree of Doctor of Philosophy in the Department of Pharmacology and Therapeutics at the University of Florida. He is married to the former Carol Louise Kouka of Western Springs, Illinois, and they have two children, Rebecca Laurice and Alyson Michelle.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality as a dissertation for the degree of Doctor of Philosophy.

Betty P. Vogh

Betty P. Vogh, Chairperson
Associate Professor of Pharmacology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality as a dissertation for the degree of Doctor of Philosophy.

C.Y. Chiou

C.Y. Chiou
Associate Professor of Pharmacology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality as a dissertation for the degree of Doctor of Philosophy.

Carl M. Feldherr

Carl Feldherr
Associate Professor of Anatomy

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality as a dissertation for the degree of Doctor of Philosophy.

David Silverman

David Silverman
Associate Professor of Pharmacology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality as a dissertation for the degree of Doctor of Philosophy.

David M. Travis

David M. Travis
Professor of Pharmacology

This dissertation was submitted to the Graduate Faculty of the College of Medicine and to the Graduate Council, and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

October, 1976

Dean, College of Medicine

Dean, Graduate School